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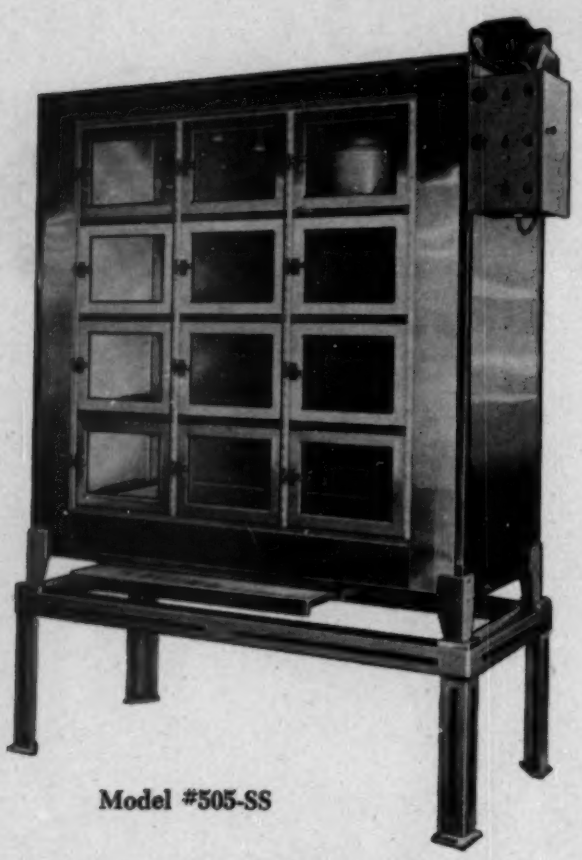

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# The Vital Story of
# WHITE FLOUR ENRICHMENT

*by* Science Writer

The blessings of better health and increased vigor are in store for many Canadians as a result of the start of flour enrichment by Canadian millers. Dramatic results in improving public health through the use of enrichment have been shown by experience in the United States, the Bataan peninsula in the Philippines, and Newfoundland. The benefits have been so outstanding in these and other nations that more and more countries are adopting enrichment.

1953 sees the enrichment of white flour put into practice throughout Canada. This program became possible through the recent amendment of the Food and Drugs Act.

Everyone in the milling industry knows that the great majority of Canadians, like other North Americans, want beautifully fine, white flour. When wheat is milled and processed to get this white flour which the public demands, vitamin and mineral values are unavoidably lost. The loss of essential vitamin and mineral elements is easily overcome by enrichment.

The enrichment of ordinary white flour is simple and inexpensive. It restores these vital vitamin and mineral factors to milled white flour: thiamine, riboflavin, niacin and iron. Calcium also may be added as an optional ingredient. Canadian specifications also provide for enrichment of higher extraction flour (Vitamin B White Flour, Canada Approved).

Technology is so advanced these days that scientists are able to "build" duplicates of many of Nature's essential complexes in the laboratory. This has happened with many vitamins. First, the chemical composition is learned and the pure substance is isolated. Then a "duplicate" is made which is identical with Nature's product chemically and in biological activity. A vitamin is a vitamin regardless of its source, just as salt is salt whether it comes from a mine or is evaporated from the sea. So efficient is large scale manufacturing that vitamins are sold at a lower cost than if they were extracted from natural sources.

These are the vitamin and mineral factors which are used in white flour enrichment:

**Thiamine**—also called vitamin $B_1$. This vitamin helps to build physical and mental health. It is essential for normal appetite, intestinal activity and sound nerves.

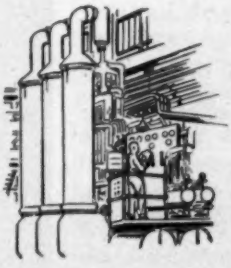
**Riboflavin**—also called vitamin $B_2$. This vitamin helps to keep body tissues healthy and to maintain proper function of the eyes. It is essential to growth.

**Niacin**—another "B" vitamin, is needed for healthy body tissues. Its use in diet is effective in controlling the serious disease, pellagra.

**Iron**—is the mineral used in enrichment. It is essential for making good red blood and preventing nutritional anemia.

To meet the amended regulations of the Canadian Food and Drugs Act, each pound of *enriched flour* must contain:

| | *Not less than* | *Not more than* |
|---|---|---|
| **Thiamine** ..... | 2.0 milligrams | 2.5 milligrams |
| **Riboflavin** ..... | 1.2 milligrams | 1.5 milligrams |
| **Niacin or Niacinamide** .. | 16.0 milligrams | 20.0 milligrams |
| **Iron** ..... | 13.0 milligrams | 16.5 milligrams |



The Hoffmann-La Roche people manufacture top quality vitamins literally by the tons. To do this they must use amazingly complex processes with scientific production controls and the latest equipment which fill a city block square and many stories high. The flour-enriching ingredients are later combined in a form called premix which flour millers add to flour manufacture so that the enrichment is spread evenly throughout the flour.

This article, reprints of which are available without charge, is published as a service to the milling industry by the Vitamin Division, Hoffmann-La Roche Inc., Nutley 10, New Jersey. In Canada: Hoffmann-La Roche Ltd., 286 St. Paul Street, West, Montreal, Quebec.

# OF COURSE,
# IT IS INCORRECT

to try to read the "baking value" of a flour out of a Farinogram. In the first place, there is no agreement at all on what constitutes "baking value" in a flour. To a baker or test baker used to medium strong flours, a really strong flour would have an inferior baking value. He would probably undermix it, underferment it and generally "underabuse" it and would produce a "green" loaf. Vice versa, the baker used to strong flours would probably overmix and over-abuse a medium strong flour and produce an inferior loaf. However, good bread could have been made from both flours with proper handling.

Test baking, in other words, is a highly subjective "art" and can never become a scientific basis for flour evaluation. Instead, the scientific approach is to determine **separately** those many factors which together make up the conglomerate we call "baking value".

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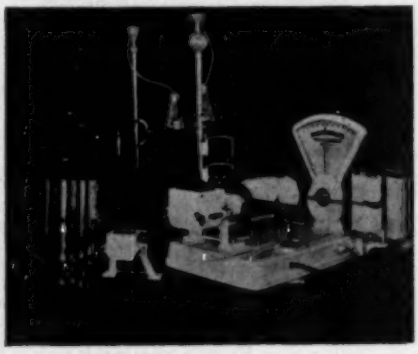

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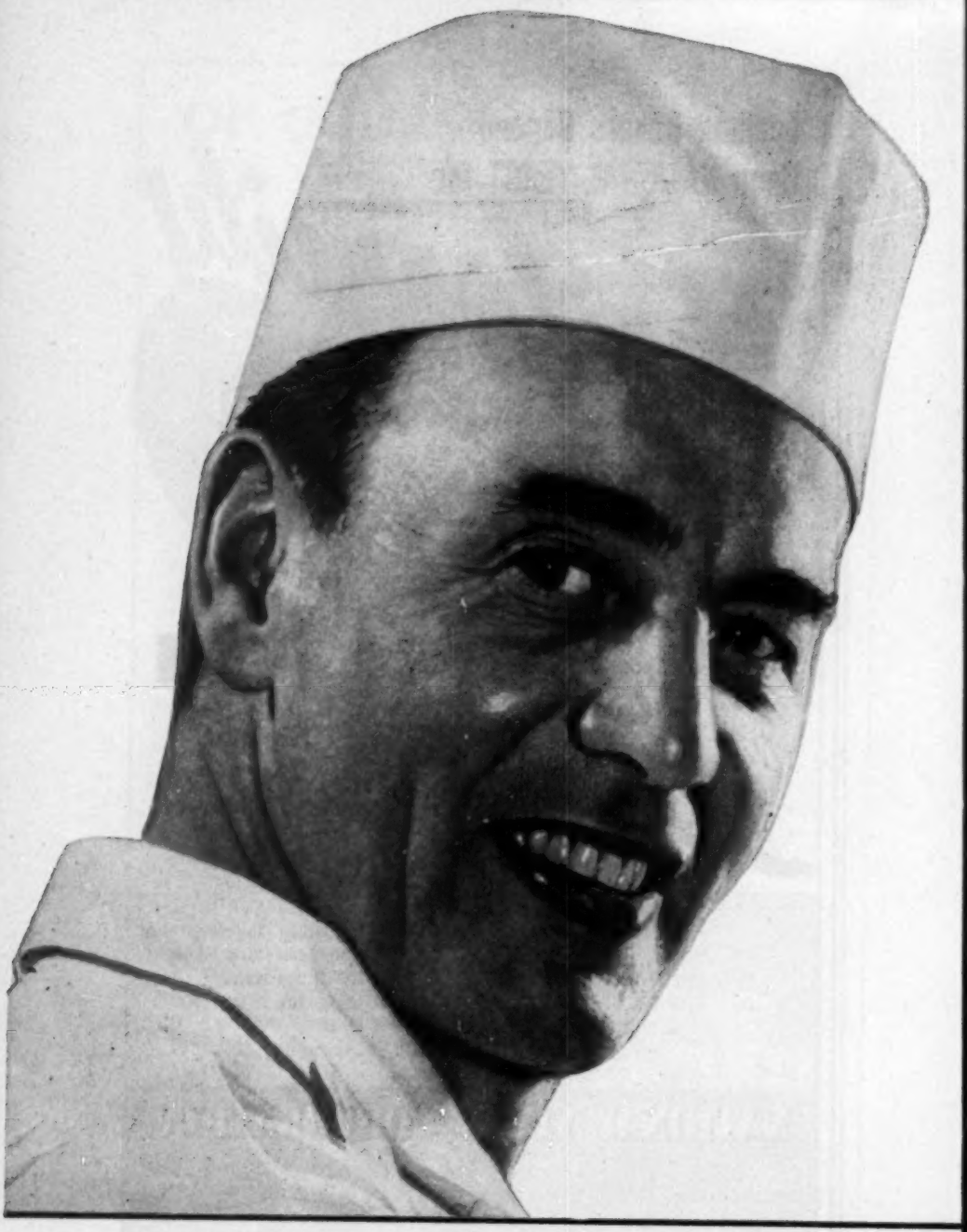

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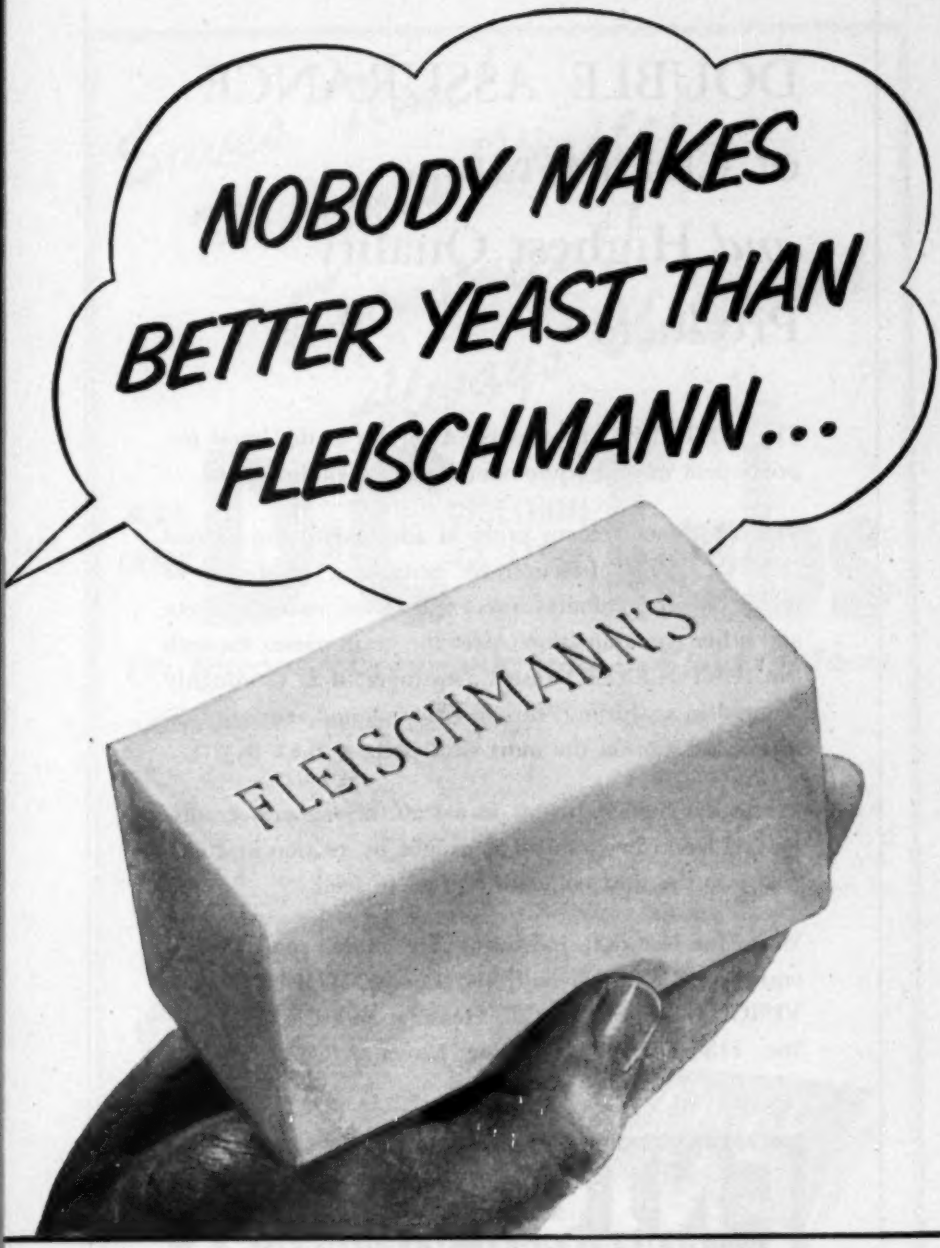


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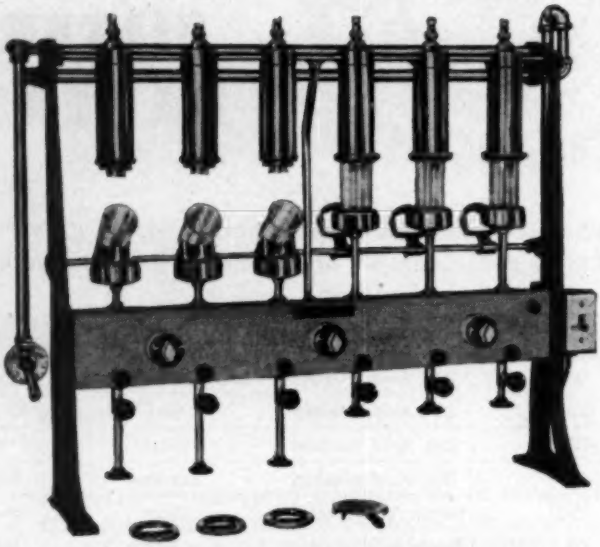

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CEREAL CHEMISTRY

VOL. XXX JULY, 1953 No. 4

# THE ALBUMIN AND GLOBULIN PROTEINS OF WHEAT[1,2]

JAMES W. PENCE AND ANGELINE H. ELDER

## ABSTRACT

Purified samples of the albumin and globulin proteins of wheat were prepared, and certain physical and chemical properties were determined by electrophoretic, ultracentrifugal, osmotic pressure, and chemical examination.

Albumins were extracted from flour with a dilute phosphate buffer and subsequently separated from contaminating gliadin and pentosans by ammonium sulfate fractionation. Globulins were extracted with 1 *M* sodium chloride and were purified by fractional precipitation with ammonium sulfate and dialysis.

The albumin proteins consisted of at least six individual components similar in molecular size but differing in electrophoretic properties. The albumin group is characterized by a tryptophan content considerably higher than that of other wheat proteins and by a low amide-nitrogen content. The apparent average molecular weight of the albumins in dilute salt solutions was about 28,000, but in the presence of urea or sodium salicylate it decreased to about 17,000.

Three individual globulin components were found in preparations made from flour and de-fatted wheat germ. A high-molecular weight component was present which is believed to correspond to the $\delta$-component found by Quensel (18) in barley but reported to be absent from wheat by Danielsson (6). The globulins are characterized chemically by low tryptophan and amide-nitrogen contents and by a high arginine content. The amide and tryptophan values were not significantly different in preparations containing various ratios of individual components.

Since the classical work of Osborne (15), numerous attempts have been made to isolate the soluble proteins of wheat or to determine their relative amounts, but knowledge concerning the occurrence and properties of these proteins is meager. Such knowledge prior to 1944 has been reviewed by Bailey (2). In general, fractions were classified by the ready coagulability of albumins and the solubility of globulins in dilute, neutral salt solution. Few areas of agreement with respect to the composition or quantities of the soluble proteins in wheat

[1] Manuscript received November 18, 1952.

[2] Contribution from Western Regional Research Laboratory, Albany, Calif., Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U.S. Department of Agriculture.

were found by various investigators. Estimates of the soluble protein contents of flours range from about 12 to 60% of the total flour protein. Recent demonstrations of the importance of soluble proteins in the baking performance of reconstituted doughs (8, 17) have led to renewed interest in the characteristics and quantities of these proteins in flours.

Traditionally, the soluble proteins of wheat were believed to consist of an albumin, a globulin, and a poorly defined material called "proteose" by Osborne (15). That such a simple picture is not correct is shown by the report of Danielsson (6), who found wheat to contain two distinct globulin components by means of ultracentrifuge examination of salt extracts of flour. Similar globulins were also found in the seeds of other species of the Gramineae family of grasses. Quensel (18) designated the four globulin components of barley as $\alpha$-, $\beta$-, $\gamma$-, and $\delta$-components according to increasing molecular weight, and Danielsson (6) retained the some notation. The embryo of wheat was found to contain only the $\gamma$-component, but endosperm contained chiefly the $\alpha$-component with smaller amounts of the $\gamma$-globulin. Bran contained more of the $\gamma$-component than of the $\alpha$-component.

The multiplicity of the albumin proteins, likewise, can be inferred from results published by Laws and France (12), who observed the appearance of several components during electrophoresis of distilled-water extracts of flour. Recent work in this Laboratory and elsewhere[3] has confirmed the occurrence of several protein components in the water-soluble fraction of flours.

Investigations were therefore begun to determine the number of components among the soluble proteins, their principal characteristics, and their influence upon the baking properties of flours. Although separation of individual components has not been achieved, considerable information about these proteins was obtained. This paper is an account of the preparation of samples of the albumin and globulin groups of proteins and a preliminary characterization, by electrophoretic, ultracentrifuge, osmotic-pressure, and chemical methods, of the materials obtained.

## Materials and Methods

A commercial, unbleached flour milled principally from soft wheats was used for the preparation of both albumins and globulins, but globulins were also prepared from a sample of commercial wheat germ defatted with petroleum ether (boiling point 40°C.).

[3] Personal communications from Drs. Wm. F. Geddes and J. R. Coffman.

Sedimentation experiments were conducted in a Model E Spinco Ultracentrifuge[4] (manufactured by the Specialized Instruments Corp.) described elsewhere (19). Moving boundary electrophoresis experiments were conducted in a modified Tiselius-Klett apparatus. Electrophoresis on paper was conducted according to the method of Kunkel and Tiselius (11), using Schleicher and Schüll paper No. 598. Osmotic pressure measurements were obtained essentially as described by Bull and Currie (5).

Tryptophan was determined by the method of Spies and Chambers (20), arginine by the method of Brand and Kassell (3), and pentosans by a modification of the methods of Brown (4) and Drury (7). Amide nitrogen was determined by the method described by Mecham and Olcott (13).

*Preparation of Albumins.* Details of the method of preparation used for the albumins are as follows: Flour was extracted with distilled water or with 0.1% phosphate buffer (pH 6.8), using 4 ml. of extractant for each gram of flour. The suspension was stirred or shaken at room temperature for 30 to 40 minutes, and the solids were removed by centrifuging. The supernatant was concentrated under vacuum to about one-fourth of its original volume. Solid ammonium sulfate was then added, with stirring, to a concentration of 0.4 *M*, and the pH was adjusted to 6.0 to 6.2, if necessary. After about an hour, the precipitate was removed by centrifuging and discarded. The main protein fraction was precipitated by adding ammonium sulfate to a concentration of 1.74 *M* at pH 6.0 to 6.2. After at least two hours of standing, the precipitate was collected by centrifuging and the supernatant was discarded. The precipitate was taken up in enough water to give a concentration of about 0.4 *M* ammonium sulfate, assuming half the volume of the precipitate to be of 1.74 *M* concentration. A final precipitation with 1.74 *M* ammonium sulfate was then conducted. The product was washed twice with small portions of 1.8 *M* ammonium sulfate, dialyzed in cellophane tubing till free of salts, and dried by lyophilization. A yield of 3 to 5 g. was obtained from each kg. of flour used.

The protein material extracted from flour by water or very dilute buffer solutions (for instance, 0.1% phosphate buffer at pH 6.8) ordinarily contains 12 to 16% of its total nitrogen in the form of amide nitrogen. These values suggest the occurrence of significant amounts of gliadin in the extracts, since Osborne (15) and Jones and Gersdorff (10) found both albumin and globulin to contain less than 10% of their total nitrogen in the amide form. It is also known that gliadin

[4] Mention of trade names of materials and equipment does not constitute endorsement by the Department of Agriculture.

is soluble to a certain extent in water and in dilute sodium chloride solutions. In view of this, efforts were made to remove gliadin from extracts of flour by means of precipitation with low concentrations of ammonium sulfate. Preliminary results showed that relatively small amounts of protein, high in amide-nitrogen, could be precipitated by concentrations of ammonium sulfate up to 0.8 *M;* above this concentration larger amounts of protein containing less amide-nitrogen were precipitated. The results in column 6 of Table I show that a high amide-containing fraction, presumably gliadin, can successfully be removed from a 0.1% phosphate-buffer extract of flour by 0.4 *M* ammonium sulfate without precipitating significant amounts of other proteins.

TABLE I

THE SEPARATION OF ALBUMINS FROM A pH 6.8, 0.1% PHOSPHATE-BUFFER EXTRACT OF FLOUR BY FRACTIONAL PRECIPITATION WITH AMMONIUM SULFATE

| Fraction | Yield[1] | Original N In Fraction | Composition of Fraction | | |
|---|---|---|---|---|---|
| | | | N | Pentosans | N as Amide N |
| | | % | % | % | % |
| **First precipitation** | | | | | |
| 0–0.4 *M* ppt. | 7.5 | 24.6 | 14.5 | 2.7 | 21.0 |
| 0.4–1.74 *M* ppt. | .... | 49.3 | ... | ... | ... |
| 1.74–3.64 *M* ppt. | 20.0 | 23.3 | 4.2 | 71.5 | 7.6 |
| **Second precipitation[2]** | | | | | |
| 0.4 *M* ppt. | 1.8 | 4.4 | 13.7 | 2.8 | 9.6 |
| 0.4–1.74 *M* ppt., $H_2O$-insol. after dialysis | 1.5 | 40.7 | 15.3 | 0.8[3] | 8.6 |
| 0.4–1.74 *M* ppt., $H_2O$-sol. after dialysis | 7.9 | | 16.9 | 1.3[3] | 8.3 |

[1] Yields are g. of material from 3 kg. of flour from which 4.3 g. of N were extracted.
[2] Reprecipitation of first 0.4-1.74 *M* ppt.
[3] Combined fractions contained approximately 3% pentosans before washing twice with 1.8 *M* ammonium sulfate.

Flour contains approximately 1% of water-soluble pentosans which are difficult to separate from the soluble proteins (16). Table I shows that a partial separation of these materials also can be accomplished with ammonium sulfate. Relatively small amounts of pentosans are precipitated by 1.74 *M* ammonium sulfate, whereas approximately two-thirds of the protein remaining in the 0.4 *M* supernatant is thrown

down. A second precipitation between 0.4 and 1.74 *M* ammonium sulfate removes most of the pentosans contaminating the main protein fraction, and by washing the final precipitate with 1.8 *M* ammonium sulfate, a nearly pentosan-free product is obtained. Successful separation of the protein remaining with the pentosans has not yet been achieved. It is not known whether any specific interaction occurs between this protein and pentosans. The solubility limit of the pentosans in ammonium sulfate seems to be fairly sharp, because increasingly larger amounts precipitate as the concentration of the salt is raised gradually above 1.74 *M*.

The small amount of protein which becomes insoluble during dialysis of the final precipitate (line 5, Table I) exemplifies a sensitivity of the albumins to denaturation which was commonly encountered during work with these fractions. Solutions of the albumins were found to be more stable in the cold (4°C.) or in the presence of salt (0.1 *N* sodium chloride).

The pH at which the above precipitations were conducted was found to have only a minor influence on the efficiency of the separations, but slightly better results were obtained between 6.0 and 6.5. Extracts were customarily concentrated under vacuum to about one-fourth their original volume (starting with 4 ml. of liquid per gram of flour) before precipitations were made in order to take advantage of a greater efficiency of separations in more concentrated solutions.

*Preparation of Globulins.* According to the method of Quensel (18), globulin components are separated by fractional precipitation with ammonium sulfate. The fraction precipitating at 15% of saturation is rich in the $\alpha$-component, found predominantly in the endosperm of wheat; the fraction precipitating at 40% of saturation contains more or less unresolved, polydisperse material; and the fraction precipitating at 70% of saturation contains both $\alpha$- and $\gamma$-globulins, but is predominantly rich in the $\gamma$-component, found chiefly in the embryo tissue.

Details of the globulin preparation method are as follows: Flour or defatted wheat germ was extracted with 4 ml. of 1.0 *M* sodium chloride (buffered at pH 7.0 with 0.1 *M* phosphates) for each gram of starting material by stirring for 1 to 2 hours at room temperature. The solids were removed by centrifuging and discarded. The supernatant was made 15% saturated by adding solid ammonium sulfate with stirring (saturation was considered to be equivalent to 760 g./liter of water at room temperature). After standing at least an hour, the precipitate was removed by centrifuging. A second and third fraction were similarly obtained from the supernatant by successive

precipitations at 40% and 70% of saturation with ammonium sulfate. The final supernatant was discarded. The precipitates obtained at 15% and 70% of saturation were taken up in water and dialyzed for at least 24 hours after the dialysate was free of salts; the resulting precipitates were extracted with either 0.2 or 0.5 *M* sodium chloride (buffered at pH 7.0 with 0.05 *M* phosphates) and again dialyzed against distilled water for at least 24 hours after the dialysate was free of salts. The precipitated globulins were dissolved a second time in the salt solution and re-precipitated by dialysis before being dried by lyophilization. The volume of salt solution used to extract the dialyzed ammonium sulfate precipitates was usually about one-tenth that used for extraction of the starting material.

The amide-nitrogen content of the protein material extracted from flour with 1.0 *M* sodium chloride at pH 7 is usually between 9 and 12% of the total nitrogen, indicating that even at this high salt concentration, significant amounts of gliadin are present. However, gliadin, albumins, and water-soluble pentosans can be removed from globulins by dialysis during which the globulins precipitate, and the other materials, for the most part, remain in solution and are discarded. Impurities which precipitate with the globulins usually fail to dissolve when the globulins are again taken up in salt solutions for reprecipitation. This is illustrated by the results shown in Table II for globulins prepared from wheat germ. The tryptophan and

TABLE II

THE SEPARATION OF GLOBULINS FROM A 1.0 *M* SODIUM CHLORIDE EXTRACT OF DEFATTED WHEAT GERM BY FRACTIONAL PRECIPITATION WITH AMMONIUM SULFATE AND DIALYSIS

| Ammonium Sulfate Fraction | Sodium Chloride Fraction[1] | Yield/kg. Germ | N | N as Amide N | N as Tryptophan N |
|---|---|---|---|---|---|
| | | g. | % | % | % |
| | 0.2 *M* NaCl-sol. | 0.8 | 17.7 | 6.4 | 1.00 |
| 0–0.15 sat'd. | 0.2 *M* NaCl-insol. | 5.7 | 15.0 | 5.7 | 1.70 |
| | 0.2 *M* NaCl-sol. | 5.1 | 17.8 | 6.4 | 0.99 |
| 0.15–0.40 sat'd. | 0.5 *M* NaCl-sol. | 2.1 | 18.4 | 6.4 | 0.83 |
| | 0.5 *M* NaCl-insol. | 10.6 | 16.6 | 5.8 | 1.45 |
| | 0.2 *M* NaCl-sol. | 9.5 | 18.2 | 6.0 | 0.81 |
| 0.40–0.70 sat'd. | 0.2 *M* NaCl-insol. | 0.5 | 14.9 | 6.3 | 2.02 |

[1] The ammonium sulfate precipitates were taken up in water, dialyzed free of salts, and the supernatants discarded. The precipitates were then extracted with 0.2 *M* sodium chloride followed by 0.5 *M* sodium chloride. The fractions soluble in 0.5 *M* salt for the 0.15 and 0.70 saturated precipitates were less than 200 mg. in amount.

amide contents of the salt-insoluble residues suggest the presence of unidentified material in wheat germ, since values for similar fractions obtained from flour indicate that denatured albumin is the principal contaminating material.

Care must be exercised to continue dialysis until complete precipitation of the globulins has occurred because Quensel (18) observed that certain components were slow to precipitate. Dialysis was usually continued for at least 24 hours after the dialysate was free of salts.

## Properties of the Protein Preparations

*Sedimentation Behavior.* Ultracentrifugation of a purified sample of flour albumins in 0.1 *N* sodium chloride at pH 6.3 showed only the one boundary seen in Fig. 1. Although the peak is not as sharp as those obtained with homogeneous materials, the broadening is mod-

Fig. 1. Ultracentrifuge (Spinco) analysis of a 0.89% solution of purified wheat albumins in 0.1 N sodium chloride at pH 6.3. The direction of sedimentation is to the left, and the photograph was taken 85 minutes after the start of the run.[5]

erate and indicates a fair degree of homogeneity with respect to molecular size of the particles present. The sedimentation constant, $S_{20,H_2O} = 2.54$ Svedberg units, is equivalent to a molecular weight of at least 21,000, since no correction for any hydration or dissymmetry of the molecules was made.

Sedimentation diagrams obtained with globulin preparations showed the presence of various ratios of $\alpha$- and $\gamma$-globulins, and in most instances, a small amount of a heavier component thought to be $\delta$-globulin. No $\beta$-globulin was found in any of the preparations. Typical diagrams are shown in Fig. 2.

The $\delta$-globulin component found by Quensel (18) in barley was reported to be absent from wheat by Danielsson (6), but in most of

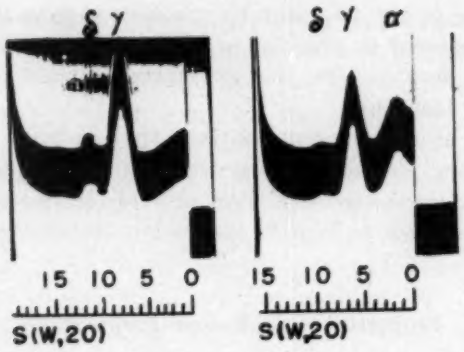


Fig. 2. Ultracentrifuge (Spinco) analyses of approximately 1.5% solutions of purified wheat globulins in 0.5 *M* sodium chloride buffered at pH 7.0 with 0.05 *M* phosphates. The direction of sedimentation is to the left. The photograph at the left shows the γ- and δ-components of globulins prepared from wheat germ (duration of sedimentation 51 min.). The photograph at the right shows the α- and γ-components, with a small amount of the δ-component, prepared from flour (duration of sedimentation 47 min.).

our preparations from flour at least a trace of a component appeared whose position in the diagrams and sedimentation constant led us to believe it to be δ-globulin. In preparations from germ, as much as 20% of this component appeared. The sedimentation constants found for the various components are shown in the following table along with those given by Danielsson for wheat globulins and those by Quensel for barley globulins. All the values for the γ-component have been corrected for the concentration dependence found by Quensel ($s = 8.30 - 0.6c$, where c is the percentage concentration of protein and 8.30 is the sedimentation constant at $c = 0$). The sedi-

| *Component* | *Sedimentation constant, $S_{20}$*[1] | | |
|---|---|---|---|
| | *Present study* | *Danielsson* | *Quensel* |
| α | 2.22 | 2.5 | 2.49 |
| β | ... | ... | 6.21 |
| γ | 7.65 | 8.7 | 8.30 |
| δ | 10.64 | ... | 12.0 |

[1] Given in Svedberg units = $10^{-13}$ sec. and referred to water at 20°C.

mentation rates obtained in the present study are all between 8 and 12% lower than those reported by the Swedish workers. This experience is quite similar to that of Miller and Golder (14), who obtained sedimentation constants for four crystalline proteins which were 7 to 11% lower than those found by various investigators using the oil-turbine ultracentrifuge in Svedberg's laboratory but which were in

close agreement with values reported by other workers in this country and abroad. Possible causes for the discrepancies were discussed. It was concluded that the *type* of centrifuge and the particular samples of proteins were not responsible. With regard to the present study, therefore, our results agree with those of previous workers as to the identity and sedimentation properties of the globulin components studied.

*Electrophoretic Behavior.* In contrast to the near-homogeneity shown by a purified albumin preparation in the ultracentrifuge, electrophoresis by the free-boundary method of Tiselius showed the preparation to be grossly heterogeneous electrophoretically (Fig. 3). In

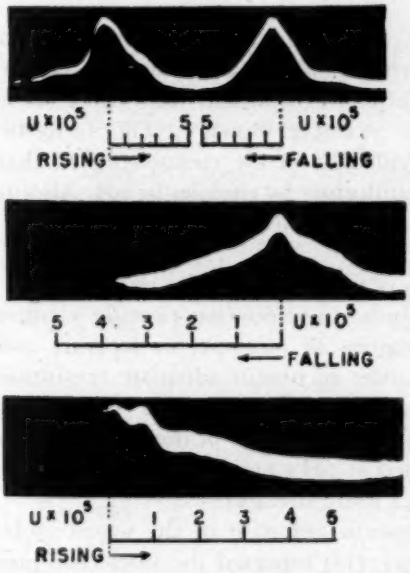


Fig. 3. Electrophoretic analysis of a 0.83% solution of purified wheat albumins in 0.08 *M* sodium chloride-0.02 *M* sodium cacodylate at pH 6.0 (26°C.) and an ionic strength of 0.1. The upper photograph was taken at 7000 seconds and the lower photographs at 19,000 seconds.[5]

spite of the number of possible components visible in Fig. 3, the electrophoretic properties of the main components are quite similar, in general, since approximately 5 hours were required to obtain the resolution shown. The graduated scale[5] in the photographs indicates

[5] The graduated scales included in Figs. 1, 2, and 3 are merely for convenience in comparing results obtained under different conditions and do not represent explicitly the relationship between points on the scale and points on the refractive index gradient curve. Such relationships are complex and are described elsewhere (1, 21).

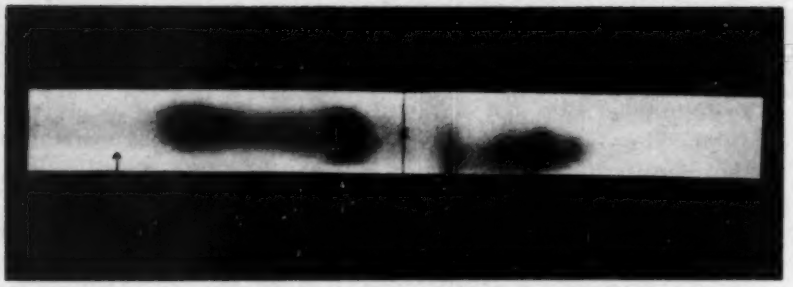

Fig. 4. Electrophoretic analysis of a solution of purified wheat albumin on filter paper. The buffer was 0.08 *M* sodium chloride–0.02 *M* sodium cacodylate at pH 6.1, and an ionic strength of 0.1. The cross indicates the origin, and the arrow at the left indicates the extent of movement of the pattern due to the electro-osmotic flow of water–hence the position of zero electrophoretic movement.

that the mobility of the main components is rather low at the pH used. Electrophoresis on filter paper showed the presence of at least six separate components, of which only three seem to be of major magnitude in the example shown in Fig. 4. Results at other pH's support the individuality of the components in that their migration was not affected uniformly by changes in pH. Also, preparations from different flours showed different amounts of the individual components.

The simplicity of electrophoresis on paper and the electrical heterogeneity of the albumin components provide a convenient means for following the progress of attempts to separate individual albumin components. In order to obtain adequate resolution of components, it was necessary to reduce the pentosans content of preparations to a level below 10%. Best resolution of the albumin components on filter paper was obtained at pH's between 6 and 8 using cacodylate or barbital buffers at 0.1 ionic strength.

The electrophoretic behavior of the wheat globulins was not investigated. Quensel (18) reported the isoelectric points of the $\alpha$- and $\gamma$-components to be 5.0 and 5.7, respectively, at an ionic strength of 0.1 in acetate and phosphate buffers. He found that the electrophoretic distribution of barley globulin components was similar to that obtained by sedimentation.

*Osmotic Behavior.* A few molecular weight determinations were made from osmotic pressure measurements of purified albumin preparations dissolved in various media. The values are summarized in the following table:

The marked decrease in molecular weight of the albumins dissolved in urea or salicylate was accompanied by a certain degree of reversibility. If the salicylate or urea was removed by dialysis and

| *Medium* | *Apparent average molecular weight* |
|---|---|
| 0.1 *M* sodium chloride, pH 5.9 | 28,000 |
| 15% sodium salicylate, pH 6.9 | 17,000 |
| 10 *M* urea, 0.05 *M* phosphate, pH 7.7 | 17,000 |

replaced with 0.1 *M* sodium chloride, a portion of the material precipitated, and the molecular weight of the soluble portion was again about 27,000. Since as much as 30 to 40% of the protein became insoluble during this treatment, it appears that changes other than simple dissociation were also caused by the two well-known denaturing agents.

TABLE III

PARTIAL AMINO-ACID COMPOSITION OF PURIFIED ALBUMIN AND GLOBULIN PREPARATIONS OBTAINED FROM WHEAT BY VARIOUS INVESTIGATORS

| Factor | Present Study | Osborne (15) | Jones and Gersdorff (10) | Hoffman and Gortner (9)[1] |
|---|---|---|---|---|
| **Albumins** | | | | |
| Total N | 17.1 | 16.8 | 15.4 | 16.7–13.5 |
| Amide N[2] | 8.3 | 8.4 | 7.7 | 10.7–10.3 |
| Tryptophan N[2] | 2.60 | ... | 4.2 | .... |
| Arginine N[2] | 16.3 | 11.4 | 20.7 | 15.7–10.7 |
| **Globulins** | | | | |
| Total N | 18.6 | 18.4 | 17.7 | 14.8–18.3 |
| Amide N[2] | 6.0 | ... | 5.9 | 8.3–10.6 |
| Tryptophan N[2] | 0.73 | ... | 2.2 | .... |
| Arginine N[2] | 29.5 | ... | 25.3 | 14.3–18.6 |

[1] The results in this column are for two preparations made by different methods.
[2] Results expressed as per cent of total N.

*Chemical Properties.* Certain of the analytical features of composition of the soluble protein preparations obtained are shown in Table III along with values reported by previous investigators. In general, the values agree better with those found by Osborne (15) and Jones and Gersdorff (10) than those of Hoffman and Gortner (9).

By comparing results for the two classes of proteins, it is apparent that the globulins have a significantly greater nitrogen content than the albumins, but the latter contain a definitely larger amount of

amide-nitrogen. An outstanding and characteristic feature of the albumins is a tryptophan content that is more than three times as large as that of the globulins. On the other hand, the globulins have an arginine content nearly double that of the albumins.

It is noteworthy that globulin preparations containing widely different ratios of components (see Fig. 2) were not significantly different in amide and trytophan contents, but a preparation containing about 50% $\alpha$- and 50% $\gamma$-globulin had a significantly lower arginine content than preparations containing less $\alpha$-globulin. Albumin preparations which were shown by electrophoresis to contain different ratios of components also were uniform in amide-nitrogen and tryptophan contents, although the variations in the ratios of components were not so pronounced in this case.

## Discussion

Classification of the soluble wheat proteins as either globulin or albumin has heretofore been based primarily on solubility characteristics. These criteria have not been satisfactory because clean separations were not obtained and results varied when different salt solutions were used (2). Results obtained in the present study now provide additional and more reliable criteria of identification which are based on several characteristic physical and chemical properties. It seems reasonably certain that the major components of each group have been detected, although other components probably will be found, particularly among the albumins, since pentosans have yet to be separated adequately from a portion of this group. Other components, however, are not apt to occur in amounts sufficient to alter significantly the general characteristics of the main groups as now known.

The uniformity of the values found among preparations having different ratios of components suggests that the amide-nitrogen and tryptophan contents of the individual major components will not differ materially from the average values already obtained. These two characteristic features of composition of the two types of soluble proteins are being made the basis for a method of estimating the total albumin and globulin contents of flours.

The occurrence of the $\delta$-component in globulins prepared in this laboratory may indicate inherent differences in domestic wheats and those examined by Danielsson (6) in Sweden. This possibility plus the quantitative variation in albumin components in some flours, as mentioned above, suggests a field of investigation which may yield

information of much interest with regard to problems pertaining to quality variation among flours and to wheat breeding.

**Acknowledgments**

The authors are indebted to Wilfred Ward and Dale Smathers of this Laboratory for the ultracentrifuge and the Tiselius-electrophoresis runs and to Ali Mohammad for the osmotic pressure determinations. The technical assistance of Eli Weinstein is likewise gratefully acknowledged.

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# EFFECT OF BISULFITE AND THIOGLYCOLIC ACID ON THE VISCOSITY OF WHEAT GLUTEN DISPERSIONS[1]

DOYLE C. UDY[2]


## ABSTRACT

Viscosities of stabilized gluten dispersed in dilute acetic acid and in 8% solutions of sodium salicylate have been measured as a function of the protein concentration before and after the addition of small amounts of sodium bisulfite or thioglycolic acid. For dilute acetic acid dispersions, the logarithm of the relative viscosity is a linear function of protein concentration in the range of 1 to 5% protein. An extension of the linear plot intersects the ordinate at a positive value, and the slope is dependent on the varietal source of gluten. After addition of sodium bisulfite or thioglycolic acid or for dispersions in 8% sodium salicylate, the linear relationship holds for concentrations of less than 1% protein. Also, the straight line passes through the origin, and the slope is independent of the varietal source of gluten. These characteristics are attributed to repression of the electrostatic forces of interaction between the protein molecules by the high ionic strength medium when dispersed with sodium salicylate, and to the rupture of disulfide bonds along with repression of electrostatic forces when sodium bisulfite or thioglycolic acid is added.

Lyophilizing or heating the dispersions altered the gluten, as evidenced by viscosity measurements in dilute acetic acid. In contrast, dispersion viscosities in sodium salicylate and in dilute acetic acid with sodium bisulfite or thioglycolic acid added were not affected by prior heating or lyophilizing of the gluten.

It is suggested that the differences in viscosity of wheat glutens are closely associated with the electrostatic forces prevalent in the native gluten. Furthermore, the intrinsic viscosity data indicate that for all wheat glutens the constituents of the complex which contribute to the viscous behavior of gluten dispersions are very nearly the same size.


Stability of wheat gluten dispersions and the pronounced effect of reducing agents on the dispersed gluten, as first described by Olcott *et al.* (9), have prompted the present investigation. According to the work cited above, a short heat treatment of the gluten dispersion eliminates proteinase activity and gives a stable dispersion as measured by viscosity and other methods. Also, the addition of small quantities of various reducing agents, including sodium bisulfite, brought about a rather sharp decrease in viscosity, which approached a minimum value after a few hours and then remained unchanged.

Measurement of the stable viscosity values of gluten dispersions

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Contribution from the Western Wheat Quality Laboratory, Division of Agricultural Chemistry, Washington Agricultural Experiment Stations, The State College of Washington; and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.
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before and after addition of reducing agents, and a study of the dependence of viscosity on the concentration of gluten dispersions under different conditions, are reported in this paper. The minimum viscosity values resulting from treatment of gluten dispersions with reducing agents have been found to be equal for the glutens from several varieties of wheat.

An attempt is made to explain the changes in the behavior of dispersions of gluten on treatment with reducing agents by regarding the dispersion as a protein polyelectrolyte in which dissociation of disulfide cross-linkages plays an important role.

## Materials and Methods

Before the method is described which was finally adopted for preparing gluten dispersions for viscosity measurements, a brief discussion of how lyophilizing and a short heat-treatment affects the gluten preparation may be of interest.

*Effect of Lyophilizing.* When crude gluten is lyophilized from dilute acetic acid dispersions to a moisture level of 2 or 3%, it can be ground and stored for an apparently indefinite period without change—as evidenced by subsequent viscosity measurements of the gluten in an 8% sodium salicylate dispersion. Dispersions of gluten in sodium salicylate have been reported (1, 9) as being stable with respect to viscosity without prior heating. Although the rate of viscosity decrease was found to be much less in this solvent than in dilute acetic acid, inactivation of the enzymes responsible for proteolysis is apparently incomplete. If the dispersion is heated in boiling water (96.5°C.) for 15 minutes, the slow decrease in viscosity originally present is completely stopped, and constant viscosity readings are maintained over several days' time.

Independent preparations of lyophilized gluten from the same flour did not yield replicable viscosity values for dispersions in dilute (0.05*M*) acetic acid, whereas sodium salicylate dispersions of this same gluten were completely reproducible as shown in Table I in the following section. A possible reason for this is the influence on electrostatic properties of the protein molecules by the high ionic strength of sodium salicylate dispersions. When dispersed in dilute acetic acid, the protein ions can exert an electrostatic effect on the viscosity. Lyophilizing apparently causes some change in the protein-charge relationship insofar as it affects the dispersion viscosity.

In order to minimize the time that the gluten was dispersed in dilute acetic acid prior to being heat-treated and during which time

proteolysis takes place, the dispersion was heated before lyophilizing. This, however, did not yield glutens whose subsequent dispersions were stable with respect to viscosity. Although the gradual decrease in viscosity that results may suggest the possibility of partial reactivation of the proteinase enzyme by lyophilizing, further study of this phenomenon is necessary.

When freshly prepared wet gluten was dispersed directly without having been previously dried by lyophilizing, either dilute acetic acid or sodium salicylate as the dispersing medium gave reproducible viscosity data (see Table I). Consequently, this procedure was adopted.

*Effect of Heating.* The initial viscosity value of dispersions of gluten in 8% sodium salicylate was not affected by heating for 15 minutes in water at the boiling point. In contrast, those dispersions in dilute acetic acid underwent a uniform decrease in viscosity when heated, as shown in Table I.

TABLE I

RELATIVE VISCOSITY VALUES[1] OF ELGIN GLUTEN DISPERSIONS[2] AT 3.20% PROTEIN CONCENTRATION AS AFFECTED BY VARIOUS TREATMENTS

| Dispersion Medium | Lyophilized | Heated[3] | Neither Lyophilized nor Heated |
|---|---|---|---|
| Acetic acid (0.05*M*) | 3.35<br>3.25<br>2.92<br>2.91<br>2.78<br>2.52 | 2.69<br>2.63<br>2.60 | 3.24<br>3.22<br>3.19 |
| Sodium salicylate (8% solution) | 2.71<br>2.69<br>2.68<br>2.65 | 2.69<br>2.65<br>2.64 | 2.70<br>2.68<br>2.63 |

[1] Measurements were made before any appreciable proteolysis.
[2] Each value is a determination from an independent preparation of gluten from the same flour.
[3] Heated 15 minutes in boiling water.

Heating for longer periods caused an even greater reduction in the viscosity value relative to the initial viscosity of the non-heated dispersion. It should be emphasized that any given heat-treatment stabilizes the gluten dispersion with respect to any further viscosity decrease caused as a result of proteinase activity.

*Gluten Preparation.* The foregoing preliminary observations led to the use of the following procedure for preparing gluten for viscosity measurements. Uniformly milled and blended straight-grade flours

TABLE II

DESCRIPTION OF THE FLOUR SAMPLES USED

| Variety[1] | Type | Protein | Ash |
|---|---|---|---|
| | | % | % |
| Rio | HRW | 12.4 | 1.62 |
| Wasatch | HRW | 13.0 | 1.56 |
| Comanche | HRW | 12.9 | 1.54 |
| Idaed | WS | 12.5 | 1.46 |
| Golden | WW | 10.8 | 1.56 |
| Elgin | CLUB | 10.6 | 1.50 |
| Triplet | SRW | 11.2 | 1.40 |

[1] These wheats were all grown in eastern Washington.

from the wheat presented in Table II were used in these experiments. Crude gluten was washed out of the flour with tap water in a small dough mixer and then freed of the remaining starch by dispersion in either a dilute (0.05*M*) acetic acid solution or an 8% sodium salicylate solution with a Waring Blendor[3]. After 10 minutes' centrifuging in an International Centrifuge[3] at 3,000 r.p.m., which removes essentially all of the starch, the dispersed gluten was immediately transferred into test tubes of 50-ml. capacity, stoppered except for a small air vent, and heated for 15 minutes at the boiling point of water. Next, the dispersion was quickly cooled in cold water, and aliquots were transferred by pipet to the viscometer for viscosity measurement. Dilutions were made directly in the viscometer. Gluten prepared in this way has approximately 10% residual fat. The protein content of the gluten dispersions was determined indirectly by the micro-Kjeldahl method of Keys (8).

*Measurement of Viscosity.* An Ubelohde type viscometer was made with a 100-ml. capacity bulb to facilitate dilutions in the viscometer. It had a flow time of 96.6 seconds for water at 25°C. This type of viscometer has many advantages for studying the effect of concentration upon viscosity and is discussed at some length in a paper by Davis and Elliott (2). Gluten concentrations up to 6% were prepared. The resulting flow times were in the neighborhood of 450 seconds. The relative viscosities reported here, $\eta_r$, are actually the ratio of the flow time of the gluten dispersion to the flow time of the solvent. The error is small, since the densities are not greatly different in the dilute range, and the kinetic energy correction, also small, largely cancels out. All measurements were carried out in a thermostated water-bath controlled to 25.00°C. ± 0.01°C.

[3] Mention of trade names of materials and equipment does not constitute edorsement by the Department of Agriculture.

Sodium bisulfite added to the dispersion was weighed out on an analytical balance in a small beaker and dissolved in the desired amount of heat-treated gluten dispersion before being transferred to the viscometer. Thioglycolic acid was made up in a solution of the solvent to be used. This was of sufficient concentration to permit transfer of a 5-ml. aliquot to the gluten dispersion in the viscometer which would give the desired final concentration of this reagent.

Viscosity measurements were made in duplicate on dispersions of independent preparations of gluten from the flours of several varieties of wheat. These measurements were carried out under a number of different conditions, as will be pointed out in the discussion to follow.

## Results and Discussion

It would seem likely that there are two major effects taking place when small quantities of either sodium bisulfite or thioglycolic acid are added to gluten dispersions. The first is a fairly complete repression of electrostatic effects and the second a complete reduction of all disulfide linkages. Evidence for this view is presented in the following discussion. Dispersions of gluten in sodium salicylate will be considered first, followed by a discussion of the more complicated behavior of gluten dispersed in dilute acetic acid.

*Gluten Dispersed in Sodium Salicylate.* Gluten dispersed in sodium salicylate would not be expected to show any electrostatic effects because of the high ionic strength of the medium; whereas dispersions of gluten in dilute acetic acid do exhibit electrostatic properties. These two dispersing media will, consequently, provide a means of studying the two separate effects of repression of ionic forces, and the cleavage of disulfide linkages on the protein molecules by reagents such as sodium bisulfite and thioglycolic acid.

When the logarithm of the relative viscosity, log $\eta_r$, is plotted against the protein concentration for gluten dispersed in 8% sodium salicylate, a linear relationship is obtained. Furthermore, the straight line passes through the origin, and it is somewhat surprising that the slopes were nearly identical for all the glutens examined from six different varieties (Fig. 1).

The intrinsic viscosity value of these gluten dispersions can be obtained from the data in Fig. 1 by using the relationship $[\eta] = \lim_{c \to 0} \frac{\ln \eta_r}{c}$ where $[\eta]$ is the intrinsic viscosity and c is expressed in g./100 ml. of solution. Since the log $\eta_r$ *vs.* concentration plot has an intercept of

zero, the $\frac{\log \eta_r}{c}$ values are constant, and the intrinsic viscosity could be calculated from a single determination at any concentration. A more reliable intrinsic viscosity value is obtained, however, by extra-

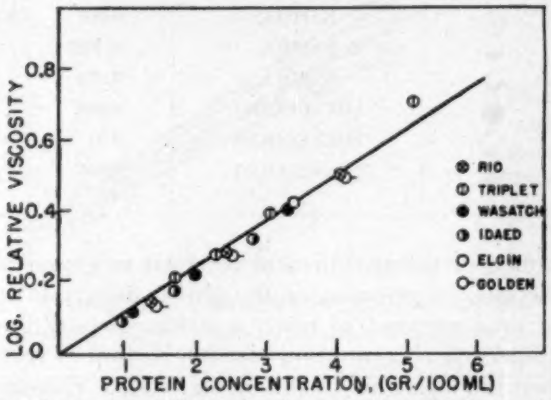


Fig. 1. Dependence of the relative viscosity on protein concentration for gluten from several varieties dispersed in 8% sodium salicylate solution.

polating and utilizing all the data. For the above dispersions of gluten in sodium salicylate a value of 0.309 was obtained.

With reference to the modified Staudinger expression, $[\eta] = KM^a$, which relates the molecular weight, M, to the intrinsic viscosity, where K and $a$ are constants for a given molecular species, the data in Fig. 1 imply a close similarity in the size of the gluten constituents for these dispersions for all wheat varieties.

*Effect of Sodium Bisulfite and/or Thioglycolic Acid.* When either

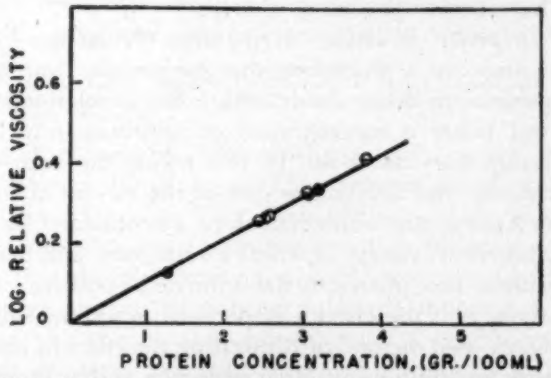


Fig. 2. Effect of reducing agents on the relative viscosities of gluten from several varieties dispersed in 8% sodium salicylate. See table below for symbol identification and experimental conditions.

| Variety and Symbol | Reducing Agent | Conc. (molarity) | pH |
|---|---|---|---|
| Triplet ⊕ | $NaHSO_3$ | 0.005 | 5.5 |
| Triplet ⊖ | $NaHSO_3$ | 0.005 | 6.6 |
| Rio ◒ | $NaHSO_3$ | 0.0025 | 6.6 |
| Rio ◒ | $NaHSO_3$ | 0.005 | 9.5 |
| Golden ● | $HSCH_2COOH$ | 0.005 | 6.8 |
| Golden ○ | $HSCH_2COOH$ | 0.01 | 6.8 |
| Golden ⊗ | $HSCH_2COOH$ | 0.005 | 4.3 |

sodium bisulfite or thioglycolic acid is added to gluten dispersed in sodium salicylate, the viscosity of the gluten dispersion immediately drops. The time required to reach a stable viscosity level depends upon the dispersion concentration and the amount of reducing agent added. When the logarithm of this stable relative viscosity is plotted against the protein concentration, a linear plot as shown in Fig. 2 is obtained. It is interesting to note that the slope is independent of the reducing agent used, the concentration of this reagent within certain limits, the pH of the dispersion between 4.3 and 9.5, and the varietal source of gluten. The slope represents an intrinsic viscosity value of 0.248 for these reduced gluten dispersions.

Since the slope is independent of the concentration of the reducing agents within certain limits, it is reasonable to expect that all the susceptible disulfide linkages are broken. Also, the independence of the slope with pH indicates that the electrostatic forces of the protein molecules have been repressed or altered.

*Gluten Dispersed in Dilute Acetic Acid.* When the logarithm of the relative viscosity is plotted against the protein concentration for gluten dispersions in dilute acetic acid, a linear relationship is again obtained, but below a concentration of approximately 1% protein the relationship does not hold. In this region the relative viscosity falls off gradually and approaches that of the solvent at infinite dilution. Figure 3 shows that a different slope was obtained for the gluten from each individual variety of wheat investigated, and that an extension of the linear plot intercepts the ordinate at positive values rather than the origin, as is the case for dispersions in sodium salicylate.

A commonly used method of illustrating the effect of concentration upon viscosity for solutions of large molecules is that shown in Fig. 4 where $(\ln \eta_r)/c$ is plotted against the concentration. Here it is seen

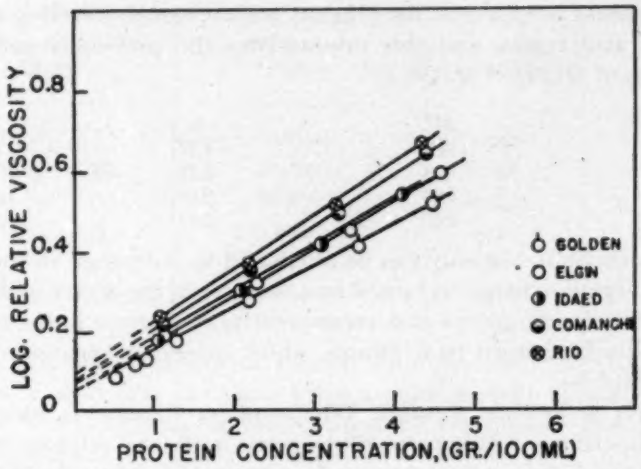


Fig. 3. Dependence of relative viscosity on protein concentration for gluten from several varieties dispersed in dilute (0.05*M*) acetic acid.

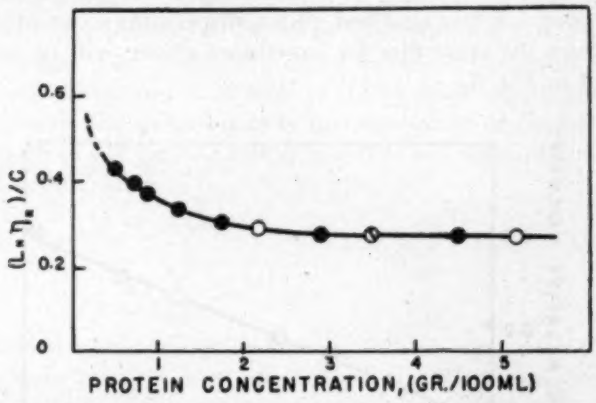


Fig. 4. Effect of protein concentration on the reduced viscosity, $\ln\eta_r/c$, illustrating the upsweep at low concentrations which is characteristic of polyelectrolytes. The data are for gluten from Golden wheat dispersed in 0.05*M* acetic acid. Different symbols represent independent preparations; pH = 4.20.

that the relative viscosity rises rapidly as the concentration is decreased in the very dilute region. This prevents the determination of a finite intrinsic viscosity. This characteristic is well known in the behavior of polyelectrolytes, and much evidence has been accumulated to indicate that the phenomenon is associated with the dissociation of ionizable groups on the polymer molecule followed by repulsion between the like charges and, consequently, a straightening out or uncoiling of the large molecule (4, 6). This leads to an increase in viscosity of the solution.

As would be expected, the viscosity is increased by lowering the pH in the acid region and this substantiates the previously published findings of Olcott *et al.* (9).

| *pH* | $\eta_r$ |
|---|---|
| 3.02 | 3.41 |
| 3.74 | 3.19 |
| 4.05 | 3.05 |
| 4.30 | 2.57 |

This increase in viscosity can be attributed to a decrease in the number of negative charges as caused by a decrease in the degree of dissociation of the acidic groups and, consequently, an increase in the number of positively charged basic groups, which causes an expansion of the molecular coil.

*Effect of Sodium Bisulfite.* When sodium bisulfite is added to a 6% dispersion of gluten in dilute acetic acid, the relative viscosity rapidly decreases and reaches a minimum value after a few hours. If the logarithm of the relative viscosity is plotted against the protein concentration for this modified gluten dispersion, a considerable difference from the same plot for unreduced gluten will be noticed, as evidenced in Fig. 5.

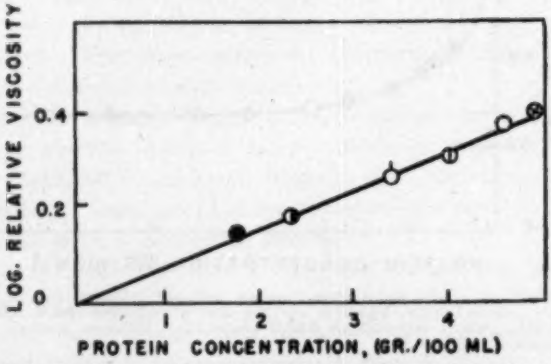


Fig. 5. Effect of sodium bisulfite and thioglycolic acid on gluten dispersed in 0.05*M* acetic acid. See table below for symbol identification and experimental conditions.

The slope values are the same, within narrow limits, for all the gluten preparations obtained from several different varieties of wheat. Also, the reproducibility of the slope value for completely separate gluten preparations from the same flour sample is quite satisfactory. The intrinsic viscosity value obtained from these data, 0.188, is lower than that obtained for gluten dispersed in 8% sodium salicylate. This indicates that the protein molecules are more expanded in the sodium salicylate medium and this is the better dispersing agent. For each

| Variety and Symbol | Reducing Agent | Conc. (molarity) | pH |
|---|---|---|---|
| Golden ○ | $NaHSO_3$ | 0.005 | 3.9 |
| Wasatch ● | $NaHSO_3$ | 0.005 | 4.2 |
| Rio ⊗ | $NaHSO_3$ | 0.0025 | 3.9 |
| Triplet ◫ | $HSCH_2COOH$ | 0.01 | 5.2 |
| Idaed ○ | $HSCH_2COOH$ | 0.01 | 3.6 |
| Elgin ◑ | $HSCH_2COOH$ | 0.005 | 3.6 |

of the two concentrations of sodium bisulfite and thioglycolic acid used, it is seen that the slope value is independent of the concentration. This parallels the results obtained for similar treatment of gluten dispersed in 8% sodium salicylate.

Another striking feature of the effect of sodium bisulfite on the form of the log $\eta_r$ *vs.* protein concentration plot is that the linear relationship now holds over the entire concentration range and intersects the origin. This is the same as was observed for gluten dispersed and reduced in sodium salicylate. It is also quite significant to note that the gluten dispersion viscosity is independent of the pH, at least in the region of pH 3.6 to 5.2 after addition of either sodium bisulfite or thioglycolic acid.

| *pH* | $\eta_r$ |
|---|---|
| 3.6 | 2.04 |
| 3.9 | 2.06 |
| 4.2 | 2.01 |
| 5.2 | 1.98 |

A minimal concentration of sodium bisulfite that would still give the same slope was not determined. However, if the concentration of sodium bisulfite is increased to 0.01*M* in a 6% gluten dispersion, a new phenomenon enters the picture. The same minimum value for the relative viscosity is obtained for a given protein concentration, but it does not remain at a fixed level. Instead, it begins to increase until the dispersion forms a gel (Fig. 6). An explanation for this has been proposed by Pence and Olcott (10). They have presented evidence which indicates that the reducing agent causes fragmentation of the protein molecules by rupturing the disulfide cross-linkages. It is also postulated that the reduced protein molecules undergo denaturation with subsequent associations—probably through hydrogen bonding of their sulfhydryl groups. As evidence, they state that gelation does not occur in the presence of urea nor if the sulfhydryl groups are alkylated with iodoacetamide.

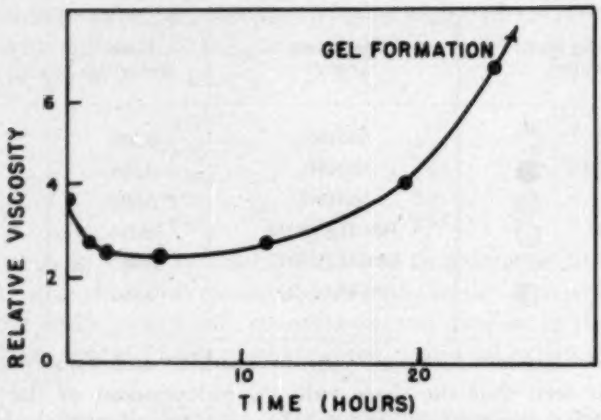


Fig. 6. The effect of sodium bisulfite on a 6% dispersion of gluten in dilute acetic acid which has been heat-treated and where the bisulfite concentration has been increased to 0.01M.

From the foregoing results it is possible to write an empirical equation which will hold for the data obtained for protein concentrations greater than 1%. The case of gel formation is, of course, an exception, since this very probably arises from phenomena other than that which we are considering here. The equation can be written as follows,

$$\frac{\ln \eta_r}{c} = k - \frac{B}{c},$$

where k is a constant, $B = 2.3\ b$, and b is the intercept of the straight line obtained from the log $\eta_r$ *vs.* protein concentration plot. This equation will also demonstrate qualitatively the curves of the data plotted above for protein concentrations less than 1%. For example, when b is finite and not zero $(\ln \eta_r)/c$ becomes infinite as the concentration approaches zero. At high protein concentrations, the last term becomes small and $(\ln \eta_r)/c$ approaches the constant value k. In all cases where b is zero, the last term is zero, $(\ln \eta_r)/c$ is constant, and a finite value for the intrinsic viscosity is obtained.

The experimental results would seem to support the hypothesis that the charges on the protein molecules in dispersion, as well as the disulfide cross-links, are affected by the sodium bisulfite or thioglycolic acid. The relationship depicted in Fig. 4 would indicate that dispersed protein molecules in the absence of reducing agent are capable of dissociating, and that there is some interaction of the resulting charges at the lower concentrations. In the presence of a reducing agent, the protein molecules behave as though they were free of these electro-

static forces, since the relationship as shown in Fig. 5 is satisfied down to zero protein concentration. Also, since the pH of the reduced dispersion has little, if any, effect on the relative viscosity under these conditions, it is apparent that the interaction of ionic forces on the protein molecules have been fairly well checked.

The effects of repulsion between like charges and other ionic interactions and structural phenomena of flexible polyelectrolyte molecules, in respect to their viscosity behavior, have been the subject of recent investigations (3, 7).

As stated above, the viscosity of the non-reduced gluten dispersion increases when the pH is lowered, probably because of the increased number of positively charged amino groups which repel each other on the protein molecule. It would be expected that lowering the pH on the reduced gluten would give a similar relative increase in viscosity if the reduction merely involves the scission of the disulfide cross-linkage. Those groups which are normally ionized in acid media are also probably altered by the reducing agent, by some unknown mechanism, and thus account for the viscosity independence of the reduced gluten with respect to pH over the range indicated above.

The fact that all glutens in a given dispersing medium were decreased to exactly the same intrinsic viscosity, owing to the addition of either sodium bisulfite or thioglycolic acid within a limited concentration range, together with the fact that the difference in the intrinsic viscosity values for sodium salicylate dispersions before and after addition of the reducing agent is constant, would strongly indicate that all of the disulfide cross-links must be ruptured and that the quality of wheat gluten is not dependent upon these cross-links, since the values obtained were completely independent of the variety of wheat.

Conversely, it is suggested that the quality factor is associated more intimately with the electrostatic forces prevalent in the glutens. This would account for the dependence of the slope on variety for non-reduced acetic acid dispersions of gluten, as illustrated in Table III. When the gluten is not altered by the heat-treatment, an even higher initial viscosity is obtained—as represented by the slope values in the last column. More important, however, is the greater degree of variability of the slope between varieties as shown in the last column. These values are quite probably a closer approximation to the differences of the gluten in the native state. Alteration of electrostatic forces would also account for the denaturing effect of heating and lyophilizing on the glutens dispersed in dilute acetic acid as contrasted to no effect, by heating or lyophilizing, for the sodium-salicylate-dis-

TABLE III

EFFECT OF HEATING ON THE SLOPES OF THE STRAIGHT LINES OBTAINED FROM PLOTTING THE LOGARITHM OF THE RELATIVE VISCOSITY AGAINST THE CONCENTRATION FOR GLUTEN FROM SEVERAL VARIETIES OF WHEAT

| Variety | Slope of log $\eta_r$ *vs.* conc. plot | |
|---|---|---|
| | Dispersion heated | Dispersion not heated |
| Golden | 0.103 | 0.112 |
| Wasatch | 0.117 | 0.125 |
| Elgin-2 | 0.117 | 0.126 |
| Idaed | 0.115 | 0.130 |
| Elgin-1 | 0.118 | 0.141 |
| Comanche | 0.126 | 0.144 |
| Rio | 0.128 | 0.146 |

[1] The Elgin wheat was from different locations in different years.

persed gluten. In the latter case, the electrostatic forces are presumably swamped by the high ionic strength environment.

Although there is some question as to the validity of comparing these dispersion viscosities with true solution or colloidal solution viscosities of large molecules, an inference may be drawn from the intrinsic viscosity values that the average molecular size of the glutens from different varieties of wheat are very similar and could behave basically the same. This would lead to the very interesting possibility of treating any gluten by a controlled method to give the desired electrostatic characteristics, which may or may not be important to the gluten quality.

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# DOUGH-DIVIDER PERFORMANCE[1]

S. WALTER BUTTERWORTH[2] AND S. J. CORNFORD[3]

## ABSTRACT

The weight variations to be expected from dough dividers operating under production conditions may be estimated through the statistical analysis of test weighings. The divider error includes short term and long term factors and sampling must be conducted over a fairly long period of time. Inadequate test methods can lead to faulty conclusions concerning divider performance. "No dough time" doughs gave more constant weights than fully fermented ones. The test procedure recommended is that two consecutive pieces should be taken from every ten pieces delivered by the divider under normal production conditions and weighed to the nearest 0.1 oz. This should be continued preferably until 100 weights have been recorded. The variability of these readings about the average value is measured by the standard deviation.

Some of the most difficult technical problems of bread production in Great Britain result from the legal requirement that loaves when offered for sale shall conform to regulations governing the weight of bread. The legal position is also complicated, as is shown by the survey of this aspect of the subject in Bell's "Sale of Food and Drugs" (1). It is not proposed in this paper to deal with all the technical factors affecting the weight of bread, but rather to select and discuss what, in the view of the authors, is the most important single factor—dough divider performance. This opinion has been reached after a study of the other variables affecting bread weight, which include losses caused by processing, baking, cooling, and storage.

It is important to remember that baked products, particularly loaves of bread, are unique amongst foods in that after dividing or depositing, it is impracticable to make further weight adjustment. The weight of bread is, in fact, largely determined about halfway through the manufacturing process.

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*Variations in the Weight of Bread.* In the hope of ensuring that no loaf at the point of sale weighs less than the specified amount required by law, the weight of the dough from which the loaf is to be baked is arbitrarily adjusted to compensate approximately for the accumulated losses subsequent to the dividing process. Neither the losses nor their accumulations are exact quantities; there is always some variation and this causes the loaf weights at the point of sale to be scattered on either side of the mean value. Under ideal conditions no loaf should be even fractionally underweight and the dough weight at the divider should theoretically be such that even the occasional loaf at the extreme lower limit of the scatter should be of legal weight. This means that the average weight of loaves sold will be overweight by half the total or extreme range of the scatter or variation in the weights. This concept has proved to be true in practice when the average weight of more than 24,000 nominal 28-oz. loaves weighed at the point of sale was 28.75 oz. – an average consumer surplus of 0.75 oz. per loaf. This surplus did not ensure the absence of so-called "light-weight" loaves; that it should do so would be a fallacious assumption, and it is important to realize this.

*Dough Divider Effects.* Superimposed upon the variations in the weight of the baked bread attributable to variations in processing, baking, cooling, and storage, there are the variations resulting from the performance of the dough-dividing machine which measures dough by volume with the object of producing dough pieces of constant weight. Prolonged study of dividing machines operating under both test and production conditions has shown that faulty divider performance is more responsible for variations in the weight of bread than any other single factor.

In dealing with dough the engineer has to consider not only accuracy of weight, but also the influence of his machinery upon the rheological properties of the dough. Modern dividers "punish" or "fell" dough structure less than do older models, but the accuracy of weighing appears to have improved little, if at all, in the last half-century. About fifty years ago the degree of accuracy claimed by a divider manufacturer was plus or minus 1/3 oz. on 36-oz. dough pieces (2). In commercial practice today an over-all range of weights of 0.7 oz. may be regarded as good divider performance, but ranges four times as wide and more sometimes occur.

## Divider Performance Tests

*Measurement of Divider Performance.* With variations in performance as great as these it is clear that some agreed method of

measuring divider efficiency is desirable. Since it is equally desirable that such measurements should become an integral part of any efficient bakery routine, the test method adopted should be capable of being carried out as easily as possible and with the minimum interruption of production.

In a survey of divider performance carried out by the Technical Panel of the Federation of Wholesale and Multiple Bakers, in which the authors took part, a divider performance test was agreed upon which consisted of weighing, to the nearest 0.1 oz., upon an automatic scale, 100 consecutive dough pieces taken under normal production conditions (3). This number of pieces was selected after preliminary tests had shown that smaller numbers did not adequately reveal the performance of the dividing machine.

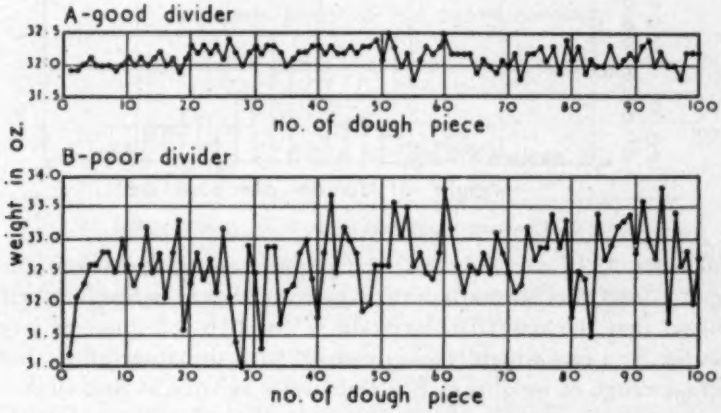


Fig. 1. Weights of 100 consecutive dough pieces.

The results of two of these tests, one on a good divider and one on a poor divider, are shown in Fig. 1, at A and B respectively. In each case, the 100 weights are plotted in their correct sequence and the difference in the variation between the two dividers is shown. The variation in Fig. 1A is from 32.5 to 31.8 oz., a range of 0.7 oz. The poor divider of Fig. 1B has given weights from 33.8 to 31.0 oz., a range of 2.8 oz. The setting of the machine was 32 oz. in each test.

*The Measure of Variation.* The frequency distributions of the weights are shown graphically in Fig. 2.

For the readings shown in Figs. 1 and 2, the standard deviations have been calculated as 0.154 oz. for the good divider and 0.563 oz. for the poor divider. These figures fix the shape of the normal curve. The position of the curve is fixed by the average value of the weights,

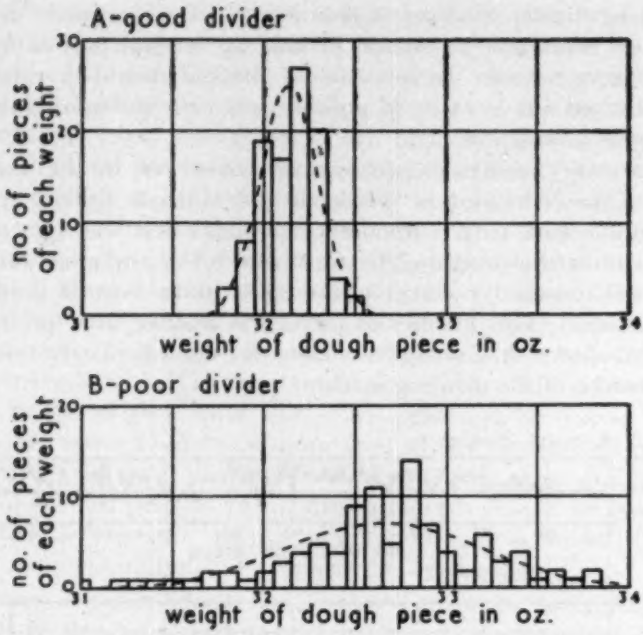


Fig. 2. Frequency distribution of the dough weights of Fig. 1.

which are 32.14 oz. and 32.64 oz. respectively. The broken line in Fig. 2 shows the normal curves based on these readings. It will be noticed that the standard deviation, s, for B is 3.7 times as large as that for A: a fact which is in agreement with the observation that the average range of weights in B is four times as large as that in A.

*Further Investigations.* In establishing a test method, it is necessary to decide on the number of dough pieces which must be weighed before the variability can be specified with sufficient accuracy for practical purposes. It cannot be done simply by weighing a few consecutive dough pieces, as misleading conclusions could be drawn, owing to the nature of the variations. This is shown by reference to an actual example.

Figure 3A shows the variations in weights of 100 consecutive dough pieces of a modern divider, working under production conditions, the operator having been warned of the test in advance and having had the opportunity of setting his machine at the required 32 oz. to the best of his ability. A comparison of section XY with section YZ in Fig. 3A indicates the differences which might be met with when shorter test groups of dough pieces are taken.

It is evident that in Fig. 3A the 100 readings vary in an irregular

manner, and in order to reveal more clearly the nature of the variations the average of the first ten readings has been calculated and plotted; this has been repeated, starting with every fifth reading, throughout the hundred. This method of obtaining averages by overlapping enables more points to be plotted than the 10 points which would be obtained by taking the average of consecutive tens, giving a smoother curve (Fig. 3B).

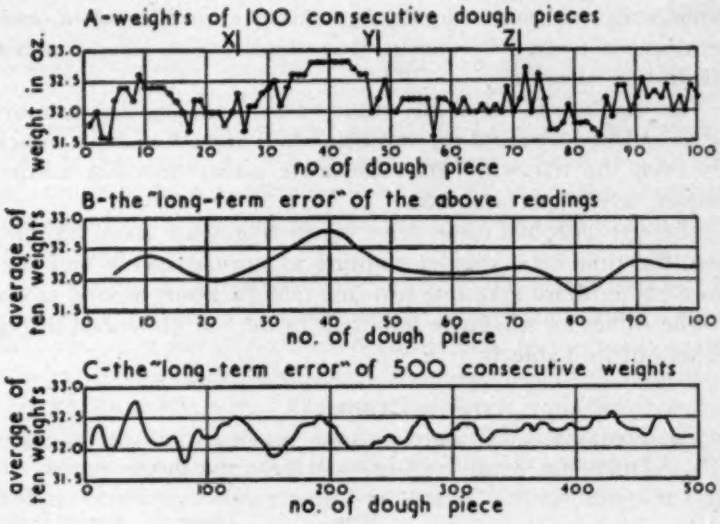


Fig. 3. Examples to illustrate the nature of the variation.

Figure 3B shows a slow variation of the average. About each of these average values the actual readings are distributed in a completely random manner. This variation about the average value is called the *short-term error*. The slow variation of the average value shown in Fig. 3B is called the *long-term error*. It is clear that over a few consecutive pieces the average value does not change by the full extent of its variation, and any estimation of error from a small number of readings must be too low.

The long-term error, which is characteristic of all dividers, is part of the intrinsic error of the divider and must be taken into account in measuring its performance. Since misleading conclusions could be drawn as a result of the number of readings being too low, it was necessary to find out whether 100 consecutive readings, normally representing an operating time of only 5 minutes, were sufficient to give an accurate picture of divider performance. Work was according-

ly carried out on groups of 500 consecutive weighings, and in Fig. 3C the averages of ten weighings are plotted for 500 consecutive dough pieces. (The calculation is identical with that used in Fig. 3B.)

Thus Fig. 3C shows the long-term error for a test run of 500 consecutive dough pieces. (Figs. 3A and 3B were plotted from the first hundred of this particular 500 test run.)

The irregular nature of the "average of tens" curve derived from 500 consecutive weighings is evident, and over such a large number of readings the variations may be regarded as entirely random, and can therefore be treated by statistical methods which assume a normal distribution of error.

The manner in which the curve varies from the first hundred to the second hundred and the third hundred and so on is an indication that even the test with 100 consecutive pieces does not adequately indicate divider performance.

The test with 500 consecutive pieces represents about 25 minutes' operating time on a divider running at normal speeds and the test covers rather more than one two-sack (560 lb. flour) mixing of dough.

The values for successive hundreds in the test illustrated in Fig. 3C are set out in Table I.

TABLE I

RESULTS OF WEIGHING 500 CONSECUTIVE DOUGH PIECES UNDER NORMAL PRODUCTION CONDITIONS WITHOUT ALTERING THE DIVIDER SETTING

| | Mean | Range | Standard Deviation |
|---|---|---|---|
| | *oz.* | *oz.* | *oz.* |
| 1st hundred | 32.18 | 1.2 | 0.31 |
| 2nd hundred | 32.25 | 1.1 | 0.24 |
| 3rd hundred | 32.15 | 1.1 | 0.20 |
| 4th hundred | 32.31 | 0.9 | 0.17 |
| 5th hundred | 32.34 | 1.2 | 0.23 |
| All 500 | 32.25 | 1.2 | 0.25 |

*Sampling.* Although the weighing of 500 consecutive dough pieces was carried out as a part of this investigation, it was realized that such a procedure would be quite impracticable as a bakery routine because too many operators are needed and production is held up. One of the facts revealed by the 500 test was that the 100 test gave useful information but that the error shown by the 100 test is normally less than the true error of the divider because the duration of the 100 test is too limited.

If the 500 test is too unwieldy and the 100 test is too short, then

TABLE II

WEIGHTS AND STANDARD DEVIATIONS OF SAMPLES TAKEN FROM 500 CONSECUTIVE WEIGHINGS

| | Dough Pieces Selected (Two from Every Ten) | | | | | |
|---|---|---|---|---|---|---|
| | 1, 2; 11, 12, etc. | 3, 4; 13, 14, etc. | 5, 6; 15, 16, etc. | 7, 8; 17, 18, etc. | 9, 10; 19, 20, etc. | All 500 |
| | oz. | oz. | oz. | oz. | oz. | oz. |
| Mean Weight | 32.28 | 32.23 | 32.26 | 32.24 | 32.27 | 32.25 |
| Standard Deviation | 0.237 | 0.262 | 0.231 | 0.258 | 0.223 | 0.246 |

some procedure, combining the virtues of both and the defects of neither, is required. The test which at present appears best to fulfill these requirements is a sampling technique in which two consecutive dough pieces are taken and weighed out of every ten pieces delivered by the divider.

The samples are weighed and returned to the production line and the sampling process is repeated until 100 weighings have been recorded, i.e., after 500 pieces have been divided. In the work reported in this paper two consecutive pieces were weighed to the nearest 0.1 oz. on an automatic scale. Eight further pieces continued normally through the process, then two more consecutive pieces were weighed. Other tests were also carried out weighing three pieces in ten. This technique is simple; it can easily be carried out by three operators working on a divider running at 24 pieces per minute or more on the types of divider commonly in use in the United Kingdom, and it does not interfere with production in any way. If the autoscale can be arranged conveniently with respect to the divider, then two operators can carry out the test at divider speeds up to 22 pieces per minute; it is necessary for one operator to record the weights obtained. It is realized that the difficulties of check weighing vary considerably with the type of divider under observation.

Table II shows the results of the "two-in-ten" technique compared with the 500 test. Samples were made up out of the 500 consecutive weighings by selecting two consecutive weights out of ten and calculating the variability. The improved accuracy compared with the 100 consecutive test is seen if the results in Table II are compared with those in Table I.

The accuracy of the standard deviation obtained by the "two-in-ten" sampling technique may be estimated from the statistical formula $s/\sqrt{2\phi}$, for the standard error of the standard deviation s, where $\phi$

is the number of degrees of freedom of s, which in this case is n-1 where n is the number of readings used in calculating the estimate of s. Substituting the value of 0.246 for s obtained by calculating from all 500 pieces, the standard error for a sample of 100 readings will be:

$$0.246/\sqrt{2 \times 99} = 0.018$$

This gives the accuracy of the estimate of the standard deviation by the "two-in-ten" method as about 7% of itself.

*Calculation of Error.* Provided a sample is taken which is evenly distributed over as many as 500 pieces, the error about the average value is sufficiently close to a normal error distribution so that it can be specified by the standard deviation, and this enables standard methods of calculation to be applied. An alternative to the tedious numerical calculation is the use of probability paper, if available. Then the inverse slope of the straight line, obtained by plotting the cumulative frequency distribution percentage of the sample versus the weight, is the standard deviation. Owing to the heterogeneity of the error, the "range" method cannot be conveniently applied.

*Interpretation.* When the standard deviation has been calculated, it is known from statistical theory that 68.3% of the values lie within $\pm$ s of the average value, where "s" is the estimate of the standard deviation, 95% of the weights lie within $\pm$1.96s and 99.8% lie within $\pm$3.09s. In terms of the number of low readings, this can be restated as meaning that 1 in 40 dough pieces will weigh less than a value 1.96s below the average and 1 in 1,000 will weigh less than 3.09s below the average.

*Reliability of the Standard Deviation for Predicting the Number of Weights Outside Given Limits.* It has been stated that about 1 in 40 dough pieces would be expected to weigh less than a limiting weight 1.96s below the average. Out of a sample of 100 weights the expectation is 2.5 pieces. The actual number obtained will depend on (a) the accuracy with which the readings conform to the normal error distribution, (b) the accuracy with which the standard deviation is known, and (c) the size of the sample.

An actual example from some results obtained using the "two-in-ten" sampling technique is given in Table III to show the accuracy attained in practice. A figure of exactly 2.5 would not be expected, since the sample is not large enough. However, the results are sufficiently accurate for all practical purposes.

*Dough Effects.* If dough were a completely homogeneous puttylike mass it might be possible to obtain greater accuracy of volume measurement and hence greater uniformity of weight. But it cannot rea-

TABLE III

COMPARISON OF ACTUAL LIGHT WEIGHTS WITH A PREDICTED NUMBER OF 2.5

| Average in Ounces $\bar{x}$ | s | 1.96 s | $\bar{x} - 1.96$ s | Actual Number Below Limits of 1 in 40 |
|---|---|---|---|---|
| 31.94 | 0.325 | 0.64 | 31.30 | 3 |
| 32.50 | 0.300 | 0.59 | 31.91 | 4 |
| 32.11 | 0.177 | 0.35 | 31.76 | 4 |
| 31.92 | 0.201 | 0.39 | 31.53 | 4 |
| 16.29 | 0.255 | 0.50 | 15.79 | 3 |
| 16.37 | 0.233 | 0.46 | 15.91 | 4 |

sonably be argued that if dough were homogeneous then divider performance would be more accurate and hence the dough is faulty and the machine is not. A dough-dividing machine must be designed and built to deal with the doughs used in commercial practice and its performance must be judged accordingly.

In order to investigate the influence of dough characteristics upon divider performance the results obtained from a divider fed with a "no-dough-time" dough have been compared with the results from the same divider fed with a fully fermented commercial dough. The "no-dough-time" dough was selected for this comparative test because it is probably the most homogeneous form of dough, free from gas pockets inflated during fermentation. The divider was known to be in good condition and giving weights as satisfactory as can be normally expected.

The "three-in-ten" sampling technique was used in order to obtain 100 samples from a single pan and the weights obtained are shown in graphic form in Figs. 4 and 5. The values derived from them are given in Table IV.

TABLE IV

NO-DOUGH-TIME DOUGH COMPARED WITH FULLY FERMENTED DOUGH

| | Bulk Fermentation Time (200 min.) | No-Dough-Time Dough | |
|---|---|---|---|
| | | Dough 1 | Dough 2 |
| | *oz.* | *oz.* | *oz.* |
| Average weight | 32.47 | 31.95 | 31.72 |
| Standard deviation | 0.17 | 0.16 | 0.18 |
| Average wt. 1–24 | 32.33 | 32.09 | 31.88 |
| Average wt. 25–51 | 32.48 | 32.02 | 31.80 |
| Average wt. 52–75 | 32.53 | 31.89 | 31.69 |
| Average wt. 76–100 | 32.52 | 31.81 | 31.51 |
| Average "s" for quarter-hundreds | 0.16 | 0.11 | 0.12 |

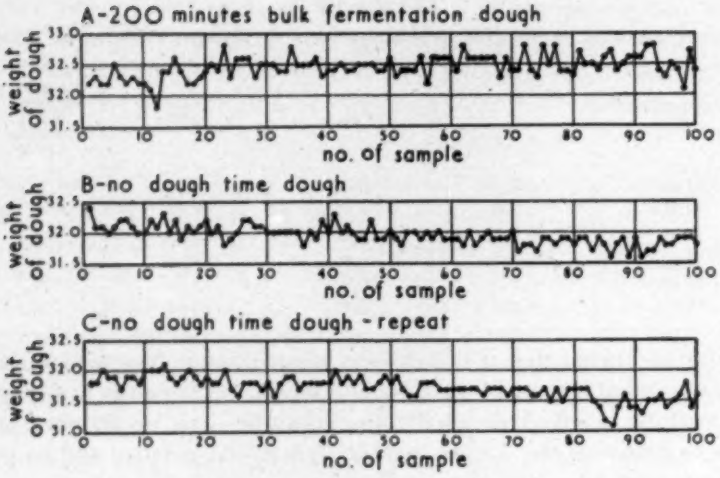


Fig. 4. Comparison of fully fermented and "no-dough-time" doughs.

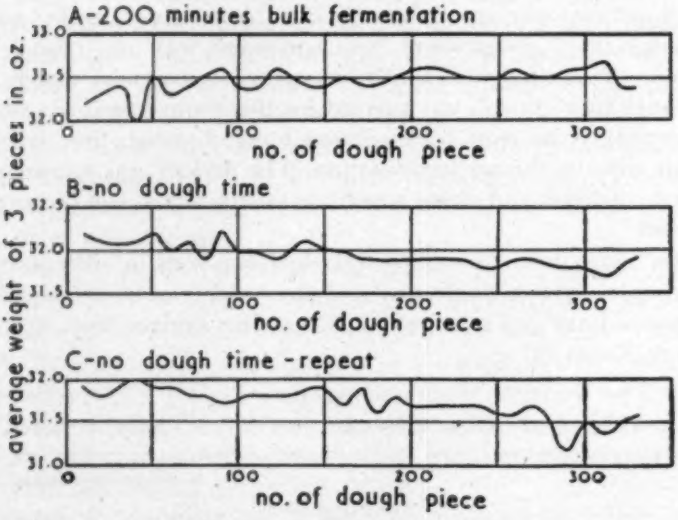


Fig. 5. The "long-term error" for the readings of Fig. 4.

Several interesting observations may be made as a result of the comparative test. As calculated from all the observations, the standard deviations appear to agree (see Table IV). However, when the readings are divided into four groups, it is seen that part of the apparent variation in the case of the no-time dough is due to a systematic decrease in the average value. The actual standard deviation for this

type of dough is significantly less, and these results show that some factor in the fully fermented dough, including its reaction to the process of dividing, is normally an important factor affecting the accuracy of dividing. The appropriate measure of the variability in this case is the variance, $s^2$, since the total variance is given by the sum of the variances due to all the different factors.

The variance for the fully fermented dough is 0.0264; the average variance for the no-time doughs is 0.0134 which is 51% of the value for the fermented dough. Thus at least 49% of the variance is due to the nature of the dough in this case. (The remaining error must be inherent in the divider mechanism.)

The factor responsible is not the consistency of the dough, since previous experiments with varying water absorption have shown that accuracy is only very slightly affected and the average value not at all.

Table IV also shows the decrease in weight of the samples as the dough starts to rise. This is due to the decreasing density, which reduces the weight of a constant volume. The total variation in weight caused by changes in density is similar to the variation in a fully fermented dough, and this suggests very strongly that the difference is due to heterogeneity in the density of the latter; this includes any heterogeneity introduced by the dividing process.

*Operational Effects.* It is customary for the operator in charge of a divider in good condition to adjust his machine to compensate to some extent for the changing character of the dough and thus obtain a higher proportion of more nearly correct weights. It should be realized, however, that the accuracy with which it is possible for the divider operator to set the average weight depends upon the accuracy of the divider itself. An inaccurate divider cannot be accurately set and it has been observed that in such cases, a divider operator weighing a few dough pieces can adjust the machine in the hope of improving the average weight and, in fact, achieve the opposite result.

*Summary of the Recommended Method.* In order to get a reliable measure of dough divider performance it has been found necessary to check the weights of pieces coming from it over as long a period as possible without alteration of the setting. It is recommended that two consecutive pieces should be taken from every 10 pieces coming from the divider under normal production conditions and weighed to the nearest 0.1 oz. This should be continued preferably until 100 weights have been recorded.

Useful information can be obtained by plotting these weights in

sequence as in Fig. 1. The long-term error shows up more clearly if the averages of the pairs are joined up by a line instead of the individual points. The variability of the readings about the average value is measured by the standard deviation.

## Discussion

The work described in this brief study of divider performance has been done with the object of discovering a satisfactory and practicable technique for estimating in a sufficiently accurate manner the weight variations to be expected from dividers operating under production conditions. It has also served to introduce a method of testing divider performance which it is hoped will enable the baking industry and bakery engineers to compare the performance of different designs of dividers and to check the state of efficiency or otherwise of dividers in constant use. The work has shown that inadequate test methods can lead to faulty conclusions concerning divider performance and divider operation. There is little need to stress the practical, legal, and economic importance of efficient dough dividing in bread production, and it is hoped that this paper may stimulate a wider interest in the subject.

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# ENZYMES THAT DEGRADE BARLEY GUMS. II. PREPARATIONS FROM BARLEY AND GREEN MALT[1]

E. J. Bass, W. O. S. Meredith, and J. A. Anderson


## ABSTRACT

Enzymes that degrade barley gums have been isolated in good yield from barley and green malt extracts by ammonium sulphate precipitation. Alcohol cannot be used as a precipitant or drying agent because it causes 90% inactivation of viscosity-reducing enzymes even at room temperature. The optimum pH for viscosity-reducing activity is 4.7 for green malt precipitates and 5.0 for barley precipitate. Iodine color tests show that amylases degrade only one fraction of barley gum, presumably the alpha-glucosan. Paper chromatography of end products of enzymatic hydrolysis indicates that malt precipitates contain an endo-beta-glucosanase and an endo-pentosanase, and barley precipitate contains at least one of these, the endo-beta-glucosanase. Malt precipates also contain enzymes that liberate glucose and one or more additional unidentified free sugars. It is suggested that the beta-glucosanase of malt is a two-component system of endo- and exo-enzymes, the second component operating by liberating cellobiose from ends of beta-glucosan chains. Cellobiose is then degraded by cellobiase to yield glucose.


The purpose of malting is modification of starch and other raw materials of barley to produce a high yield of malt extract of satisfactory quality. Since starch is potentially the greatest source of extract, it is not surprising that an impressive volume of research on the starch-degrading enzymes, the amylases, has been, and is still being, recorded. However, amylolysis may not be the only factor in starch conversion. There is reason to believe that cytolysis, the enzymatic dissolution of endosperm cell walls, must precede amylolysis. Preece (9) has reviewed some evidence favoring this view, and Dickson and Shands (3) have shown that the first step in barley modification is the dissolution of endosperm cell walls. Hence a study of cytolytic, or cell-wall-degrading, enzymes of barley and malt may contribute a great deal to the knowledge of quality in malting barley.

Barley gums prepared by Preece, Ashworth, and Hunter (10) and by Meredith, Bass, and Anderson (6) are believed to be of cell-wall origin, and appear to be good natural substrates for studying cytolytic enzymes. The greatest difficulty in preparing these natural substrates has been the danger of causing significant structural changes during extraction. This difficulty was partially overcome by Preece (10), who inactivated barley enzymes by refluxing with alcohol prior to extrac-

[1] Manuscript received January 23, 1953.
Paper No. 123 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, and No. 307 of the Associate Committee on Grain Research (Canada).

tion. However, the low viscosities of Preece's polysaccharides suggests that some degradation occurs during extraction. Sandegren and Enebo (13) use an artificial substrate; although this may be of great value in elucidating the mechanism of a single enzyme system, there may be many pitfalls in extrapolating the results to malt and malting. Meredith, Watts, and Anderson (7) have apparently avoided excessive degradation of their polysaccharides during extraction, and their methods together with those of Gilles, Meredith, and Smith (5) and Prcece and MacKenzie (12) on fractionation of barley polysaccharides may well pave the way towards preparation of natural undegraded barley substrates specific to the action of single enzymes or enzyme systems.

The present work is an extension of previous studies on bacterial gum-degrading enzymes (1) to barley and malt enzymes. The term, X-enzyme, has again been applied to all gum-degrading enzymes other than amylases. Gum-degrading enzymes have been precipitated from barley and green malt extracts and some progress has been made in identifying or classifying the enzyme systems involved in gum degradation.

## Materials

Two substrates were used in these studies. The first was obtained by alcoholic precipitation from an aqueous extract of alcohol-treated barley (7). The gum was stabilized by boiling in aqueous solution and reprecipitating with alcohol. The final product was designated "stabilized gum." The second substrate was the "papain gum" of Meredith *et al.* (7). This was prepared by alcoholic precipitation from a papain extract of alcohol-treated barley from which papain had first been removed by trichloracetic acid.

Enzyme sources were: (a) a commercial 6-day malt provided by the Canada Malting Co.; (b) Montcalm barley harvested at the University of Manitoba in 1949; (c) an experimental 6-day malt of this barley malted by the Barley Improvement Institute, Winnipeg; (d) Wallerstein's alpha-amylase preparation used in previous studies (1); and (e) Wallerstein's beta-amylase, a preparation made from selected barley.

## Methods

Determinations of enzyme activity were the same as those used in a previous paper (1). Reaction mixtures for viscosity determinations contained 7 ml. of 0.6% gum solution, 2 ml. of McIlvaine buffer

(0.1 *M* citric acid and 0.2 *M* disodium phosphate), and 1 ml. of enzyme solution.

Enzyme reaction products were examined by descending paper chromatography. Ten ml. of a 1% solution of gum were mixed with either 10 ml. of enzyme solution (barley or malt precipates) or 2 ml. of enzyme solution (Wallerstein's alpha- or beta-amylase), and 2 ml. of McIlvaine buffer solution (pH 4.5), then stored at room temperature for 24 hours. Reaction mixtures were then heated in a boiling-water bath for 20 minutes, cooled, passed through Duolite A-7 resin to neutralize acidity, and evaporated to dryness at reduced pressure. One-half to 1 ml. of distilled water was added to the dry material; the mixture was stirred, heated to boiling, cooled, and finally filtered through glass wool. About 6 to 9 $\mu$l. of concentrate were analyzed chromatographically with three different solvent mixtures: ethyl acetate, acetic acid, water (3:1:3); ethyl acetate, pyridine, water (5:2:5); and butanol, ethanol, water (5:1:4). Aniline phosphate (2) was the spray reagent.

## Precipitation of Barley and Green Malt Enzymes

*Commercial Malt.* Extracts of commercial green malt were prepared from 50 g. coarse-ground malt and 200 ml. distilled water by mashing at room temperature for 2 hours and by maceration in a Waring Blendor for 2 minutes. The room-temperature mash was filtered through E and D 509 filter paper; the blended mixture was first centrifuged, then filtered by suction through celite. Extracts obtained by the two procedures were then compared for viscosity-reducing activity. There was no significant difference in extractable viscosity-reducing activity between the two preparations, but the maceration technique was much more rapid. This procedure was therefore adopted.

Both ammonium sulphate and alcohol precipitate viscosity-reducing enzymes from green malt extract, but ammonium sulphate appears to give a much higher yield. As Preece (11) used an alcohol precipate in his enzyme studies, the efficiencies of the two precipitants were compared. The data showed that ammonium sulphate precipates at least 85% of viscosity-reducing enzymes, but alcohol apparently precipitates only about 5%. The low efficiency of alcohol cannot be due to incomplete precipitation alone, as an alcohol-washed ammonium sulphate precipitate accounts for only 4% of the total extract activity. Alcohol, therefore, appears to inactivate over 90% of viscosity-reducing enzymes of green malt at room temperature. Alpha-amylase does not seem to be similarly affected by alcohol.

As the bulk of alpha-amylase is precipitated on half-saturation with ammonium sulphate, the possibility of partial purification of viscosity-reducing enzymes by fractional precipitation with ammonium sulphate was investigated. A green malt extract was treated with an equal volume of saturated ammonium sulphate solution, and the precipitate, designated $P_1$, was removed, washed once with half-saturated ammonium sulphate solution, then dried *in vacuo* over phosphorous pentoxide. The liquor was then made 95% saturated by addition of solid ammonium sulphate, and the precipitate, designated $P_2$, was removed, washed once with saturated ammonium sulphate solution, then dried as before. The two precipitates were tested for viscosity-reducing activity and alpha-amylase activity. Precipitate $P_2$ showed 2.3 times the viscosity-reducing activity of $P_1$, but only 0.07 times the alpha-amylase activity. It may therefore be easier to purify gum-degrading enzymes of fraction $P_2$, provided that both precipitates contain all components of gum-degrading enzyme systems.

*Montcalm Barley and Malt.* Extracts of Montcalm barley and its green malt were prepared by the maceration procedure and precipitated with ammonium sulphate in two stages as before. Viscosity-reducing activities of liquors and precipitates were determined. Recoveries of viscosity-reducing activity from extracts of barley and green malt were 87% and 62%, and a 100-fold increase in activity during malting was observed.

*Activity-pH Relations.* Viscosity-reducing activities of barley and green malt precipitates were determined for values of pH between 3 and 8. Papain gum was the substrate, and McIlvaine's standard buffer solutions (0.1 *M* citric acid and 0.2 *M* disodium phosphate) were used to control pH. Figures 1 and 2 show that malt preparations $P_1$ and $P_2$ have optimum activity at pH 4.7. Figure 3 shows that barley $P_1$ has optimum activity at pH 5.0. Barley $P_2$ has little or no viscosity-reducing activity. Activity-pH curves for malt $P_1$ and malt $P_2$ are strikingly similar, suggesting that both preparations contain the same viscosity-reducing enzymes. The curve for barley $P_1$ differs from malt curves in the position of the pH optimum and in the more gradual decrease in activity at pH's above the optimum. The optimum pH for barley $P_1$ is the same as that reported for barley and malt cellulose by Sandegren and Enebo (13). It may be that the only component of the X-enzyme system in barley is a cellulase.

## Identification of Gum-Degrading Enzymes

The fractionation of methylated barley gum by Gilles, Meredith, and Smith (5) into a methyl-alpha-glucosan, a methyl-beta-glucosan,

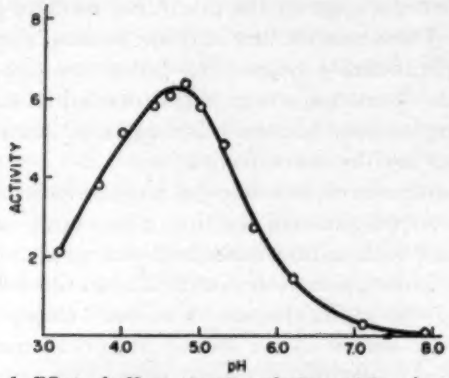


Fig. 1. Effect of pH on viscosity-reducing activity of malt $P_1$.

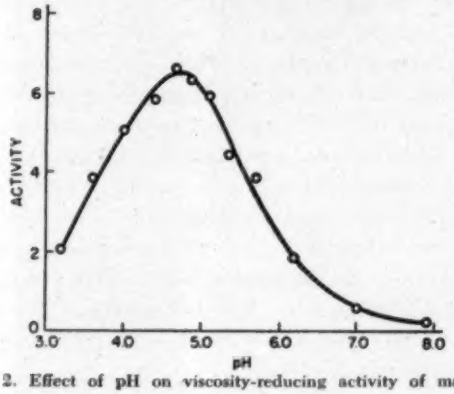


Fig. 2. Effect of pH on viscosity-reducing activity of malt $P_2$.

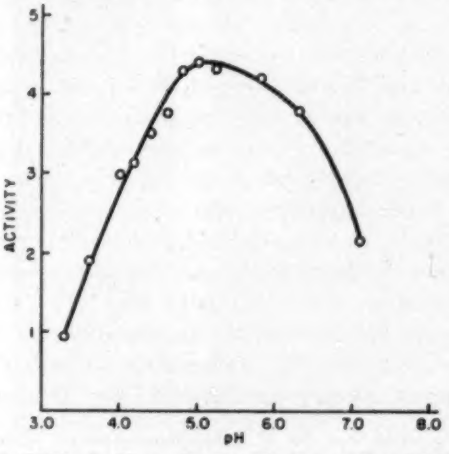


Fig. 3. Effect of pH on viscosity-reducing activity of barley $P_1$.

and a methyl-pentosan suggests the possibility of three gum-degrading enzyme systems. These may be the amylase system, a beta-glucosanase system, and a pentosanase system, the latter two presumably being components of the X-enzyme system. Confirmation of this classification of gum-degrading enzyme systems was sought by chromatography of reaction products and by other techniques.

It was readily demonstrated that the amylase system degrades only what may be an alpha-glucosan fraction. First, both substrates give a characteristic color with iodine. Stabilized gum gives a pale blue color and papain gum gives a pale violet color. These colors with iodine can be attributed to the alpha-glucosan fraction.[2] Hence the ability to form characteristic iodine colors should disappear under the action of enzymes that degrade the alpha-glucosan. The only enzyme solutions that destroy ability to give iodine colors are those containing active amylases. Others, such as the purified bacterial X-enzyme obtained from Wallerstein's alpha-amylase by chromatography (1), have no effect on iodine color. Next, the faintness of iodine colors suggest that the alpha-glucosan fraction is a minor component of barley gum. Hence enzymes that degrade this fraction should have little effect on gum viscosity. Concentrated solutions of Wallerstein's beta-amylase, Wallerstein's alpha-amylase heated at 70°C. for 15 minutes, and malt precipates heated at 70°C. for 15 minutes after addition of calcium acetate (to preserve alpha-amylase activity) do, in fact, show little or no viscosity reduction. Finally, amylases are believed to be specific to 1 : 4-alpha-glucosidic linkages, and these linkages are present only in the alpha-glucosan.

The presence of a beta-glucosanase in barley and malt was demonstrated by the action of barley and malt precipitates on Sandegren's substrate, Modocoll M (13), ethyl hydroxyethyl cellulose. Barley and malt precipitates and Wallerstein's alpha-amylase reduce viscosity of Modocoll M solutions, but enzyme concentrations 100 times those used on barley gums are necessary to cause appreciable viscosity reduction.

Valuable clues to the identity of the components of X-enzyme were obtained from paper chromatograms of enzymic reaction products. The following preparations were used as enzyme sources: a 1% solution of Wallerstein's alpha-amylase, and the same solution with alpha-amylase inactivated by storing at 0°C. and pH 3.3 for 48 hours; bacterial X-enzyme purified by chromatography; a 1% solution of Wallerstein's beta-amylase; 2% homogenates of malt $P_1$ and malt $P_2$, and the same two solutions heated at 70°C. for 15 minutes after addi-

[2] Personal communication from Dr. F. Smith, Department of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.

tion of calcium acetate; a 2% homogenate of barley $P_1$. Both substrates were used in these studies, and gave identical results.

Two types of reaction products were observed. The first type contained two or three slow-moving spots, presumably oligosaccharides. The slowest-moving spot yielded, on acid hydrolysis, arabinose, xylose, and some glucose; the other two spots yielded only glucose (7). The second type of reaction product appeared as an elongated spot covering the region from a position just above galactose up to, and including, the glucose position. This spot was attributed to glucose and one or more additional unidentified free sugars. Blanks containing enzyme or substrate alone failed to yield detectable products. Figure

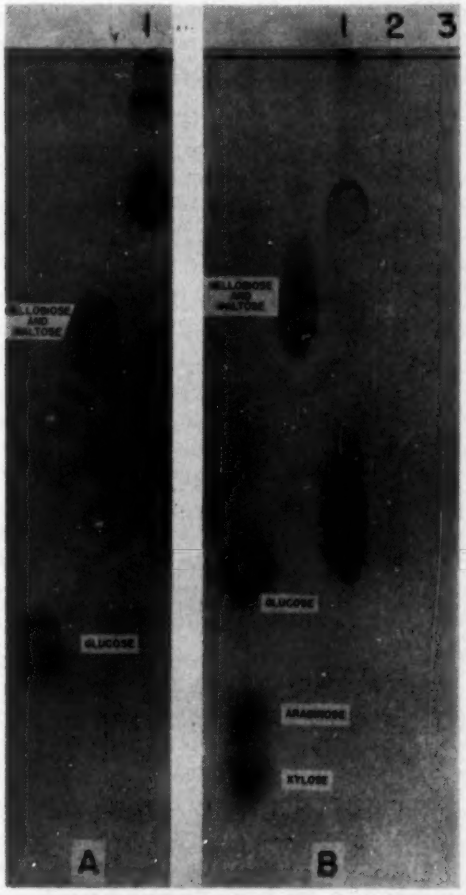


Fig. 4. Typical chromatograms of enzymic reaction products. A: 1. Stabilized gum and Wallerstein's alpha-amylase. B:1. Papain gum and malt $P_1$. 2. Papain gum and malt $P_1$, heated at 70°C. for 15 minutes after addition of calcium acetate. 3. Malt $P_1$ blank.

4A shows the three oligosaccharides obtained by the action of Wallerstein's alpha-amylase on stabilized gum. Figure 4B shows the elongated spot and two oligosaccharide spots due to the action of malt $P_1$ on papain gum.

Since both substrates yield identical enzymic reaction products, these are summarized in a single set of records. Table I shows that malt alpha-amylase and Wallerstein's beta-amylase fail to liberate detectable products, although beta-amylase yields a trace of sugar with $R_F$ equal to maltose. The failure to detect products of amylolysis suggests that the alpha-glucosan fraction may be a very small fraction of barley gum, or that this polysaccharide is degraded by amylases sufficiently to destroy ability to give iodine color but not sufficiently to yield appreciable amounts of low molecular weight products. Therefore reaction products, other than maltose, appearing on chromatograms are attributed to the action of enzymes other than amylases.

TABLE I

ENZYMIC REACTION PRODUCTS

| Enzyme Source | Enzymes Active[1] | Oligosaccharides | | Free Sugars | |
|---|---|---|---|---|---|
| | | Pentosan | Glucosan | Glucose | Others |
| Wallerstein's alpha-amylase | A X | + | + | − | − |
| Wallerstein's alpha-amylase | X | + | + | − | − |
| Purified X-enzyme | X | + | + | − | − |
| Wallerstein's beta-amylase | B | − | − | − | trace M[2] |
| Malt $P_3$ | A B X | + | + | + | + |
| Malt $P_1$ | A | − | − | − | − |
| Malt $P_2$ | A B X | + | + | + | + |
| Malt $P_2$ | A | − | − | − | − |
| Barley $P_1$ | B X | trace | trace | − | − |

[1] A = alpha-amylase; B = beta-amylase; X = X-enzyme.
[2] M = maltose.

Malt precipitates, Wallerstein's alpha-amylase, and purified bacterial X-enzyme liberate both pentosan and glucosan oligosaccharides. Thus both malt and the bacterial preparation appear to contain a beta-glucosanase and a pentosanase that hydrolyze internal linkages. Barley precipitate liberates only traces of oligosaccharide material during the 24-hour incubation period, but this is not surprising when it is recalled that barley has only about 1% of the activity of malt. Barley may therefore contain at least one of these enzymes, most likely the beta-glucosanase, as this enzyme would attack Modocoll M.

Only malt precipitates liberate free sugars, the chief component of which is glucose. Hence the beta-glucosanase system of malt can apparently hydrolyze its substrate to glucose, whereas the bacterial enzyme cannot cause such extensive degradation. A more likely expla-

nation is that malt may contain a second beta-glucosanase that attacks the ends of polysaccharide or oligosaccharide chains, releasing simple sugars. The existence of such an enzyme was suggested in a previous paper (1). As the malt precipitates are rich in cellobiase, which was demonstrated by the degradation of cellobiose to glucose, it may be that cellobiose, not glucose, is the end product of exo-beta-glucosanase action.

## Discussion

Results presented here indicate that the X-enzyme system of green malt contains at least two hydrolytic polysaccharases: a beta-glucosanase and a pentosanase. Ungerminated barley shows beta-glucosanase activity, but there is as yet no evidence for the existence of pentosanase in barley. The beta-glucosanase system of green malt appears to be a two-component system of endo- and exo-enzymes, but the second component may not be present in barley.

Since this work was completed, a paper by Enebo, Sandegren, and Ljungdahl (4) on barley and malt cellulase has been received. These authors also conclude that malt may contain at least two cellulases, the first, a "typical hydrolytic beta-polyglucosidase" corresponding with our endo-beta-glucosanase. They suggest that the second enzyme may be a nonhydrolytic transglycosidase. This is an interesting possibility that is consistent with our data, as it is conceivable that cellobiose may be the end product of transglycosidase action and glucose would appear as a result of cellobiase action.

The degradative effect of amylases on a portion of the barley gum appears to contradict the hypothesis that amylases cannot penetrate endosperm cell walls without the aid of cell-wall-degrading enzymes. However, this anomaly would disappear, and the hypothesis would be strengthened, if it could be shown that the alpha-glucosan fraction is not cell-wall material. No data are as yet available on the relative yields of this and other fractions of barley gum, but the faintness of iodine tests on papain gum suggests that the alpha-glucosan fraction is a minor constituent of the gum, and may therefore be extraneous. A similar conclusion was reached by Northcote and Horne (8), who isolated a "glycogen" fraction along with other materials of yeast cell walls, and concluded that this fraction is probably not cell-wall material.

The solution of problems such as confirmation of the existence of two-component enzyme systems in malt and the presence or absence of one or more of these components in barley will probably be facilitated by substrates specific to the action of individual enzyme systems.

Natural barley substrates obtained by gum fractionation are most desirable, as these may reflect changes that occur during malting. One such material, similar to the beta-glucosan fraction isolated by Preece and Mackenzie (12), is now being studied as a potential substrate for beta-glucosanases.

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# THE THIAMINE CONTENT OF TURKISH WHEATS AND CORRESPONDING BULGURS[1]

SAIM SARACOGLU[2]

**ABSTRACT**

The thiamine content of 28 samples of Turkish wheats from different regions ranged from 3.11 γ/g. to 5.04 γ/g. with a mean value of 3.89 γ/g. The values for the corresponding bulgurs ranged from 2.02 γ/g. to 3.57 γ/g. with an average of 2.85 γ/g.

Through conversion into bulgurs, the wheats underwent an average loss of 27.3% thiamine with the extreme values 8.9% and 42.4%. This wide variability in thiamine loss is occasioned very probably by the differences in manufacturing practices. The greatest loss occurs in the boiling process of wheat, the other steps involving little loss.

Ten samples of wheat from the Agricultural Experiment Station of Ankara contained an average of 4.45 γ/g. of thiamine varying from 3.87 γ/g. to 5.07 γ/g.

The thiamine contents are expressed on a 14% moisture basis.

Turkey is a large producer and consumer of wheat. Wheat products are the main food supply of the population; hence, the vitamin content of flour, bread, and bulgur is of great importance.

Bulgur is a wheat product having the appearance of dark-colored rice. It is difficult to give a precise definition of bulgur, because the method of its manufacture varies more or less according to the village or region where it is made. Nevertheless, the classical manufacture involves the following steps: (1) washing to remove the foreign materials and impurities; (2) boiling to gelatinize the starch; (3) air-drying, mostly effected in the sun; (4) beating in a mortar to separate the hulls; (5) fanning to remove the hulls; (6) crushing in mills; and (7) rough sieving to remove the fine part.

The good bulgurs are made of hard wheats. However, sometimes soft wheats and in certain regions even einkorn and emmer are used. The difficulty of finding wheats giving pale bulgur led to the rather common practice of dyeing them yellow. In certain localities some or all of steps 4, 5, and 7 are omitted. In boiling, the proportion often is one part of wheat to two parts of water, and the time, 1 to 1.5 hours. The evaporation of the water is compensated for from time to time. Wetting prior to beating and air-drying prior to fanning are necessary. The separation of hulls by beating and their subsequent removal by the help of the wind is not always complete. Generally there are three sieving fractions: thick bulgur, thin bulgur, and fine parts. The

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relative quantities depend upon the nature of the mill and the type of sieve used, but the fine fraction is the smallest. Thus, the bulgurs of different regions are dissimilar: they may be dyed or left undyed, coarse or fine-grained, boiled for a long or short time, and may contain a variable quantity of hulls.

The present study was undertaken to determine the thiamine content of Turkish wheats grown in different regions of the country and of the bulgurs prepared from them. Some of the intermediate products of bulgur manufacture were analyzed to determine the amount of thiamine loss at various stages.

## Materials and Methods

Twenty-eight samples of wheat and the bulgurs manufactured from them were collected in different regions of Turkey. The production years and districts are shown in Table I. The wheats comprise hard (h), soft (s), and mixed (m) varieties. One sample of einkorn and one sample of emmer are also included. The intermediate and by-products of the bulgur manufacture and ten wheat samples of the crop year 1949 from the Agricultural Experiment Station of Ankara were also investigated. The moisture content of the samples was first determined and a sieving test carried out on the original bulgurs.

The amount of thiamine was estimated by a thiochrome procedure based on that of Glick (2), with slight modifications: a weighed sample (2–2.5 g. wheat or 2.5–3 g. bulgur) was extracted with 50 ml. of 0.1 *N* hydrochloric acid (4). The extracts were filtered and 5 ml. of filtrate were used for conversion to thiochrome. Three ml. of 40% sodium hydroxide (6) without and with $K_3Fe(CN)_6$ were used (3 ml. of 1% $K_3Fe(CN)_6$ in 100 ml. NaOH). The thiochrome was extracted with 15 ml. of isobutanol, washed with 10 ml. of water (3); 10 ml. of isobutanol extract were clarified with 1 ml. of absolute ethanol and the fluorescence was measured in calibrated cuvettes with the Coleman Universal Spectrophotometer. Standardization was effected against pure thiamine solutions of appropriate concentration (4 $\gamma$/g. for wheat and 3 $\gamma$/g. for bulgur).

The values were recorded on a 14% moisture basis and represent the averages of two or more determinations which agreed within $\pm$ 5% (1, 5). The average error of all measurements was calculated as $\pm$ 3%.

## Results and Discussion

Wheats and bulgur contained an average of 9.2% moisture. The thiamine contents of the wheats and corresponding bulgurs are given

in Table I. The wheats contained from 3.11 to 5.04 γ/g. with an average of 3.89 γ/g., whereas the thiamine content of the bulgurs ranged from 1.44 to 3.57 γ/g. with a mean of 2.83 γ/g. The thiamine loss in converting wheat into bulgur varied from 8.9 to 42.4% with an average of 27.3%.

TABLE I

THE THIAMINE CONTENT OF TURKISH WHEATS AND CORRESPONDING BULGERS[1]

| District | Crop Year | Hard, Soft, or Mixed | Thiamine Content | | | Thiamine Loss | |
|---|---|---|---|---|---|---|---|
| | | | Wheat | Bulgur | | | |
| | | | | Thick | Thin | Thick | Thin |
| | | | γ/g. | γ/g. | γ/g. | % | % |
| Ankara | 1947 | h | 4.40 | 3.13 | 3.50 | 28.9 | 20.4 |
| Ankara | 1947 | m | 4.14 | 3.24 | 3.57 | 21.7 | 13.7 |
| Denizli | 1948 | h | 4.06 | 2.72 | 2.91 | 33.0 | 28.3 |
| Denizli | 1949 | m | 3.11 | 2.31 | 2.33 | 25.8 | 25.0 |
| Ilgaz | 1950 | s | 3.83 | 2.89 | 3.19 | 24.5 | 16.7 |
| Ankara | 1950 | m | 3.52 | 2.72 | 2.90 | 22.7 | 17.6 |
| Çorum | 1950 | m | 4.03 | 2.83 | 2.77 | 30.0 | 31.3 |
| Usak | 1951 | m | 3.43 | 2.22 | 2.43 | 35.3 | 29.1 |
| Ürgüp | 1951 | s | 3.96 | 2.74 | 3.00 | 30.8 | 24.2 |
| Ankara | 1951 | m | 3.87 | 2.54 | 2.80 | 34.4 | 27.7 |
| Denizli | 1951 | m | 3.17 | 2.23 | 2.02 | 29.7 | 36.3 |
| Sarayköy | 1951 | m | 4.16 | 2.58 | 2.55 | 38.0 | 38.7 |
| Ilgaz | 1951 | m | 3.73 | 3.23 | 3.39 | 13.4 | 9.1 |
| Gelibolu | 1948 | h | 3.77 | 2.79 | | 26.0 | |
| Selçuk | 1948 | m | 3.79 | 2.80 | | 26.1 | |
| Selçuk | 1948 | s | 4.65 | 2.68 | | 42.4 | |
| Gelibolu | 1949 | h | 4.20 | 2.79 | | 33.6 | |
| Sarayköy | 1949 | m | 4.14 | 2.57 | | 37.9 | |
| Denizli | 1949 | s | 3.58 | 2.98 | | 16.8 | |
| Izmir | 1950 | m | 3.70 | 3.20 | | 13.5 | |
| Çankiri | 1950 | s | 3.65 | 3.33 | | 8.9 | |
| Denizli | 1950 | m | 3.77 | 2.95 | | 21.8 | |
| Bursa | 1950 | s | 4.06 | 2.56 | | 36.9 | |
| Kayseri | 1950 | s | 4.27 | 3.17 | | 25.8 | |
| Kayseri | 1950 | s | 3.91 | 3.21 | | 17.9 | |
| Çorum | 1950 | s | 5.04 | 3.11 | | 38.3 | |
| Eskisehir | 1951 | m | 3.14 | 2.68 | | 14.6 | |
| Kilis | 1951 | m | 3.75 | 3.17 | | 15.5 | |
| Bursa | 1944 | .. | ... | 1.44 | | ... | |
| Adana | 1951 | .. | ... | 3.03 | | ... | |
| Karaman | 1951 | .. | ... | 3.15 | | ... | |
| Kastamonu | 1950[2] | .. | 2.92 | 2.41 | | 17.6 | |
| Kastamonu | 1951[3] | .. | 4.42 | 2.73 | | 38.2 | |
| Average[4] | | | 3.89 | 2.83 | | 27.3 | |
| Minimum[4] | | | 3.11 | 1.44 | | 8.9 | |
| Maximum[4] | | | 5.04 | 3.57 | | 42.4 | |

[1] Results are expressed on a 14% moisture basis.
[2] Einkorn.
[3] Emmer.
[4] The values for einkorn and emmer are not included.

In the preparation of bulgurs, the only steps in the process which result dceidedly in a loss in thiamine content are boiling and sieving.

TABLE II

THIAMINE RETENTION IN THE INTERMEDIATE AND BY-PRODUCTS OF BULGUR MANUFACTURE

| Number of Samples | Thiamine Content | | | | | | Thiamine Loss | | |
|---|---|---|---|---|---|---|---|---|---|
| | Wheat | | | Transformation Product | | | | | |
| | Min. | Max. | Mean | Min. | Max. | Mean | Min. | Max. | Mean |
| | γ/g. | γ/g. | γ/g. | γ/g. | γ/g. | γ/g. | % | % | % |
| Boiled wheats | | | | | | | | | |
| 8 | 3.19 | 4.17 | 3.80 | 2.31 | 3.73 | 2.94 | 1.2 | 40.8 | 22.6 |
| Boiled wheats without hulls | | | | | | | | | |
| 10 | 3.12 | 5.03 | 3.95 | 2.26 | 3.61 | 2.87 | 3.5 | 42.2 | 27.3 |
| Hulls | | | | | | | | | |
| 11 | 3.12 | 4.44 | 3.80 | 2.23 | 4.39 | 3.18 | | | |

As shown in Table II, the greatest loss, averaging 22.6%, occurs in the boiling step. This loss is due both to solution of the thiamine and to its destruction by heat. It is influenced by variety, by the relative proportions of wheat and water, the pH, and the duration and manner of boiling.

The removal of the hulls involved no appreciable thiamine loss. Table II shows indirectly a loss of 4.7%, but determinations with four varieties of wheat gave an average value of 2.91γ thiamine per g. for boiled wheats with hulls and an average value of 2.92γ thiamine per g. for the same boiled wheats without hulls. The weight of the hulls is negligible compared to that of the grain. The average thiamine content of the hulls amounted to 3.18 γ/g.

The sieving aims generally at obtaining three fractions: coarse (thick bulgur), medium (thin bulgur), and fine. The fine fraction is the smallest (up to 10% of the total) and is the thiamine-rich part. The amounts of the other two fractions are variable, because they depend on the type of mill and on the sieves used in different villages. The thiamine content of bulgurs increased with the fineness of the granules, as seen in Table III. It is unfortunate that the portion richest in vitamins, i.e., the fine-sieve fraction, is removed from the

TABLE III

THIAMINE CONTENT OF THE THREE SIEVE FRACTIONS OF BULGUR MANUFACTURE

| | Thick Bulgur | Thin Bulgur | Fine Part | Corresponding Wheat |
|---|---|---|---|---|
| Average thiamine content of 13 samples (γ/g.) | 2.72 | 2.87 | 3.57 | 3.81 |
| Comparison of results on a percentage basis | 71.3 | 75.4 | 93.7 | 100 |

bulgur during its manufacture. A given bulgur is richer in thiamine when it contains a greater quantity of smaller granules.

To obtain bulgur with high thiamine content, measures must be taken to avoid as much as possible lowering the thiamine level of the bulgur, or subsequently to enrich the low-content bulgur. These measures might be to start with wheat of high thiamine content, shorten the boiling time (this is possible if the wheats are softened beforehand for some hours with cold water), and omit the sieving. The possibility of an enrichment procedure is being investigated.

**Acknowledgment**

The author wishes to express his appreciation to Professors C. Dikmen and O. Gerngross for their helpful suggestions.

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# APPROXIMATE ISOELECTRIC pH's OF ALBUMINS FROM WHEAT FLOUR[1,2]

JAMES W. PENCE


## ABSTRACT

Approximate mobilities and isoelectric pH's for eleven apparently individual components of the albumin fraction of wheat flours were determined by electrophoresis on filter paper. This method gave good agreement with literature values for the isoelectric points of crystalline bovine serum albumin, ovalbumin, and beta-lactoglobulin.

The flour albumins could be divided arbitrarily into three main groups on the basis of their migration at various pH's. The range of isoelectric points for the alpha group extended from about 4.5 to 4.8, that of the beta group from about 4.9 to 5.9, and that of the gamma group from about 6.7 to 8.7. The approximate mobilities of the entire group (referred to the known mobility of bovine serum albumin) ranged from about $-2.5$ to $+1.2 \times 10^{-5}$ cm.$^2$/volt sec. at pH 6.0 and an ionic strength of 0.1.

In general, major components occurred in the beta group, whereas the gamma group was composed entirely of minor components. On the basis of relative migrations at various pH's, components of the alpha group appeared to be more closely related to one another than those of the other two groups. The gamma group appeared most diverse.

A significant variation was observed in the relative quantities of the various components in preparations from a limited number of flours.


The albumin proteins of a wheat flour were recently found to consist of a group of closely related components quite similar in molecular size but differing significantly in electrophoretic properties (5). The preliminary results indicated that at least six individual components were present in preparations which had been separated from pentosans, or other flour gums, by fractionation with ammonium sulfate. Subsequent study of similar preparations from several different flours has shown that as many as eleven apparently individual components can be detected by the technique of electrophoresis on filter paper. The present paper describes the determination of approximate electrophoretic mobilities and isoelectric pH's for these components.

## Materials and Methods

The albumin preparations were isolated from unbleached, straight-grade flours, experimentally milled from pure-variety samples of wheat (1). The varieties used principally were Turkey (Kansas) and Pentad (North Dakota), a durum variety. The albumins were sep-

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[2] Contribution from Western Regional Research Laboratory, Albany, Calif., Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

arated from contaminating proteins and hemicelluloses by fractionation with ammonium sulfate as described previously (5). Electrophoresis on paper was conducted by the method of Kunkel and Tiselius (2) with Schleicher and Schüll paper No. 598. Buffer solutions were used at an ionic strength of 0.1 and consisted of acetate, cacodylate (0.02 *M* sodium cacodylate-0.08 *M* sodium chloride), or barbital (0.01 *M* sodium diethylbarbiturate-0.09 *M* sodium chloride) systems; appropriate amounts of the corresponding weak acids were added to give the desired pH's. Electrophoresis was conducted at room temperature, with a difference of potential of approximately 4.7 volts per cm. of paper. Under these conditions, the rise in temperature due to passage of the current did not exceed 3°-5°C.

Movement of the protein patterns caused by the electro-osmotic flow of water was determined by means of xylose or other reducing sugar contained in the solution of crystalline bovine serum albumin (Armour Lot No. J4902) used as a reference protein for all runs[3]. The position of the xylose after completion of a run was detected by exposure of the paper to vapors from a solution of aniline trichloroacetate contained in an evaporating dish placed in a drying oven with the paper strips. After 15-30 minutes at 105°-110°C., a yellow-brown spot appeared at the location of the sugar. (More time was usually required for development of the xylose spot when buffers of pH's below 6 were used than when buffers above this pH had been used for the electrophoresis.) After the xylose spot was marked with pencil, the proteins were stained with bromphenol blue.

Migrations of the components were measured from the position occupied by the electrically inert xylose at the completion of the runs. Approximate mobilities were then calculated as the product of the proportional migration of each component, compared to that of the reference protein, and the known mobility of the reference protein at the same pH and ionic strength. The values used for bovine serum albumin were interpolated from mobilities reported by Longsworth and Jacobsen (4) for this protein in acetate and veronal buffers at 0.1 ionic strength.

## Results and Discussion

The reliability of the method of electrophoresis on paper, as used in the present experiments, to determine isoelectric pH's was checked by determinations with several crystalline proteins. The results of

[3] The sugar could not be added to solutions of the wheat proteins because its movement was found to be affected by the presence of the albumin components. Such interaction did not occur with the bovine serum albumin.

TABLE I

THE ISOELECTRIC pH'S OF CRYSTALLINE PROTEINS AS DETERMINED BY A METHOD OF ELECTROPHORESIS ON FILTER PAPER

| Protein | Isoelectric pH's | |
|---|---|---|
| | Found | Literature |
| Bovine serum albumin | 4.6 | 4.71 (4) |
| β-Lactoglobulin | 5.1 | 5.21 (4) |
| Ovalbumin | 4.5 | 4.58 (3) |

these measurements are shown in Table I along with values taken from the literature. Although the results are slightly, but uniformly, lower than values found by other workers, the agreement is very satisfactory.

The approximate electrophoretic mobilities shown in Table II were obtained for eleven detectable components of flour albumins at

TABLE II

APPROXIMATE MOBILITIES AND ISOELECTRIC pH'S OF ALBUMIN COMPONENTS OF WHEAT FLOUR AS DETERMINED BY ELECTROPHORESIS ON FILTER PAPER

| Component | Approximate mobility[1] in cm.²/volt. sec. x $10^{-5}$ | Approximate Isoelectric pH |
|---|---|---|
| α1 | −2.5 | 4.5 |
| α2 | −2.3 | 4.6 |
| α3 | −2.0 | 4.7 |
| α4 | −1.6 | 4.8 |
| β1 | −1.2 | 4.9 |
| β2 | −0.7 | 5.1 |
| β3 | −0.3 | 5.5 |
| β4 | −0.1 | 5.9 |
| γ1 | +0.3 | 6.7 |
| γ2 | +0.8 | 7.1 |
| γ3 | +1.2 | 8.7 |

[1] pH of 6.0 and 0.1 ionic strength in 0.02 M sodium cacodylate-0.02 M cacodylic acid-0.08 M sodium chloride. Mobility of bovine serum albumin taken as $-3.30 \times 10^{-5}$ cm.²/volt sec. (4).

pH 6.0 in a cacodylate buffer of 0.1 ionic strength (0.02 *M* sodium cacodylate, 0.02 *M* cacodylic acid, and 0.08 *M* sodium chloride). Also included in Table II are isoelectric pH's determined from the curves shown in Fig. 1 where distance of migration under standardized conditions (4.7 volts per cm. for 18 hours at 25°-28°C.) is plotted against pH. The points shown in the figure represent averages for two to four

separate runs at each pH value. In most cases, the migration values agreed within ± 1 cm.

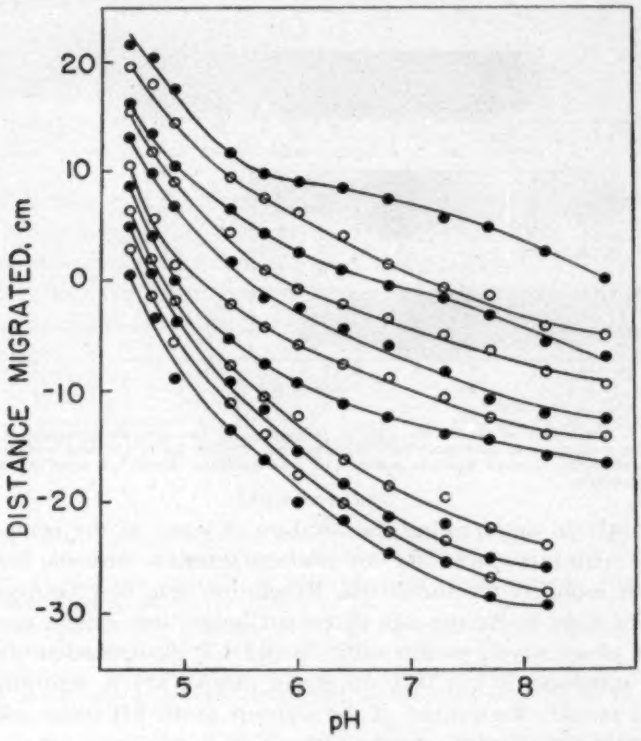


Fig. 1. The effect of pH on the electrophoretic mobility of albumin components of wheat flour in acetate, cacodylate, and barbital buffers at 0.1 ionic strength as determined by electrophoresis on filter paper.

The components were named according to the order of their mobility, and subscript designations were used to decrease the number of letters required. The arbitrary groupings were made, as much as possible, on a basis of similarity in behavior at various pH values. For instance, Fig. 2 shows that components of the $\alpha$-group migrated with much the same relative position to one another at all pH's. The behavior of the other groups was less regular, but at an intermediate pH of about 6, the grouping of these components, as indicated, appeared to be a reasonable compromise of the possible groupings shown in Fig. 2 for other pH values.

Adsorption of protein by the filter paper and inadequate resolution of the components in certain pH regions were sources of much

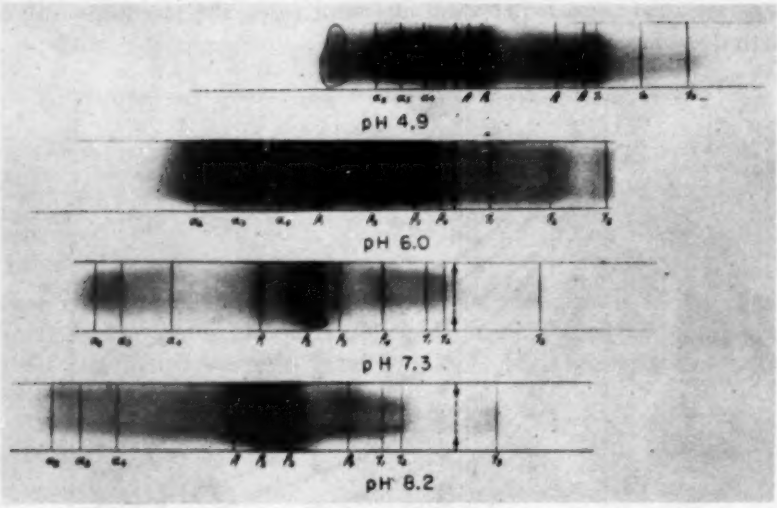


Fig. 2. The effect of pH on the relative migrations of the albumin components of wheat flour under standardized conditions (4.7 volts per cm. for 18 hours at room temperature and 0.1 ionic strength). Arrows indicate position of zero mobility. Encircled areas indicate denaturation at origin.

uncertainty in determining the location of some of the components, but the error introduced did not interfere seriously with the isoelectric point or mobility measurements. Resolution was best between pH's of about 6 to 8. As the pH decreased below this range, resolution became progressively poorer until, at pH 4.5, identification of several of the components was very uncertain. Above pH 8, resolution also became poorer. Resolution of the $\alpha$-group at all pH's was seldom as good as that of the other components.

The wide range of isoelectric points for these proteins contrasts with their general similarity in sedimentation behavior and chemical composition, insofar as the latter is reflected by amide and tryptophane nitrogen contents (5). The relatively narrow range of mobility values also is in accord with their class similarity. However, the large differences in isoelectric points of some of the components and differences in the shape of the pH-mobility curves shown in Fig. 1 imply significant differences in amino acid composition. Longsworth (3) has shown that mobility curves of a protein should be similar to acid-base titration curves, which are interpretable in terms of composition.

An approximate representation of the relative proportions of the components appears in Figs. 2 and 3 which show electropherograms for the albumins from one flour at several pH's and from two flours at one pH. The marked variation in amounts of the different com-

Fig. 3. Comparison of the relative amounts of individual components in albumin preparations from different wheat flours. Upper strip, Turkey variety (Kansas); lower strip, Pentad (durum) variety (North Dakota). Arrows indicate position of zero mobility.

ponents shown in Fig. 3 represents the extreme that was found in preparations from eight different flours of several types. Most of the patterns were much like that shown for the Turkey flour in Fig. 3. In general, the major components appear in the $\beta$-group, whereas, the $\gamma$-group is composed entirely of components occurring in minor amounts.

**Acknowledgment**

The technical assistance of Mr. N. E. Weinstein, who prepared the albumin samples used in this study, is gratefully appreciated.

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# VARIATION IN PRINCIPAL QUALITY FACTORS OF DURUM WHEATS WITH A QUALITY PREDICTION TEST FOR WHEAT OR SEMOLINA[1]

G. N. IRVINE AND J. A. ANDERSON

**ABSTRACT**

Variations of semolina pigment, semolina lipoxidase, macaroni pigment, yellow color of macaroni and macaroni color score are considerably greater with variety than with environment. Lipoxidase activity responds least to wide differences in environment, but varies fourfold among varieties. The principal factors determining macaroni color are semolina pigment and semolina lipoxidase activity. Regression equations are presented for wheat and for semolina which form the basis for a screening test using measurements of these two properties to predict the pigment content of macaroni. The test requires only 20 g. of wheat.

The objectives of this paper are two: first, to investigate the range of variation of the principal factors controlling the macaroni-making quality of durum wheats with variety and with environment; and second, to put on record a test for predicting macaroni pigment, from analyses of durum wheat or semolina, which is rapid and requires a minimum of material.

## Methods

Macaroni was processed by the micro-macaroni method (8). Pigment contents of wheat, semolina, and macaroni were determined by an overnight extraction of 8 g. of material with 40 ml. of water-saturated n-butyl alcohol; wheat and macaroni were ground at the finest setting on a Labconco mill. Macaroni color was measured with a Bausch & Lomb HSB color analyzer using special red, yellow, white, and black color discs, and color scores were calculated according to the method outlined previously (6).

Lipoxidase was determined manometrically using a Warburg apparatus; the method, based on previous studies (7), was as follows:

*Lipoxidase Assay.* Apparatus: Warburg apparatus, 15-ml. reaction vessels, water bath at 30.0°C. Stop watch.

Reagents: Phosphate buffer 0.067 *M*, pH 6.5. Substrate emulsion To 10 ml. of 60% linoleic acid add 2 ml. of Triton X-100[2]. Add dis-

[1] Manuscript received March 16, 1953.
Paper No. 127 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, and No. 306 of the Associate Committee on Grain Research (Canada). Presented in part at the Annual Meeting, May, 1953.

[2] Obtainable from Rohm & Haas Co., Philadelphia.

tilled water slowly, with mechanical stirring, until the emulsion becomes very stiff or inverts. Dilute to 250 ml. with distilled water and beat at high speed in a Waring Blendor for 3 minutes. Stopper tightly and store in a refrigerator. Emulsion will keep for 2–3 weeks.

Procedure: Grind 10 g. of semolina, flour or ground wheat, with 5 g. of sand and 20 ml. distilled water by hand or mechanically in a mortar for 3 minutes. Transfer to round-bottomed 50-ml. centrifuge tube and centrifuge at top speed in a clinical centrifuge for 10 minutes. Decant the supernatant, which constitutes the enzyme extract.

Pipette 0.8 ml. of the enzyme extract into the sidearm of a 15-ml. Warburg flask and 1.0 ml. of the substrate, with 2.0 ml. of phosphate buffer, into the main compartment. With the stopcocks open, shake at 120 oscillations per minute for about 10 minutes. Set manometer, close stopcock, and tip contents of sidearm into body of flask rinsing several times, start stop watch at moment of tipping. Determine total oxygen uptake for first 5 minutes in microliters. Activity is calculated as $\mu$l. $O_2$/min./g.

## Variation of Certain Quality Factors with Variety and Environment

*Materials.* Seven varieties, ranging from the best (Nugget) to the poorest (Golden Ball) now grown in Canada, were grown in 1952 at seven locations covering the durum-growing area of Western Canada. Semolina was milled from the 49 samples on an experimental Allis-Chalmers mill to approximately 50% extraction after purification.

*Results and Discussion.* Table I summarizes the results of the study in terms of varietal and station means for wheat yield and for various semolina and macaroni properties. The variation of wheat yield and semolina protein with environment (second section of table) gives a good picture of the wide differences between growing conditions at the seven stations.

Except for yield and protein content, all the properties, which are those connected essentially with the final macaroni color, vary considerably more with variety than with environment. This is particularly true of lipoxidase activity. The variance due to stations for lipoxidase activity is just significant at the 5% level, whereas there is a relatively enormous variance due to variety. The greater influence of variety on lipoxidase activity is also reflected in the comparisons between semolina and macaroni pigment contents. Since the latter are a function of lipoxidase activity, they show a greater variation among varieties than do the former.

The data in Table I show that two of the principal factors con-

TABLE I

STATISTICS FOR YIELD PER ACRE AND VARIOUS SEMOLINA AND MACARONI PROPERTIES

| | Yield | Semolina[1] Protein | Semolina[1] Pigment | Semolina Lipoxidase | Macaroni[1] Pigment | Macaroni Yellow Color | Macaroni Color Score |
|---|---|---|---|---|---|---|---|
| | Bu./acre | % | p.p.m. | $\mu 1.0_2$ /min./g. | p.p.m. | % | |
| **Variety Means** | | | | | | | |
| Nugget | 35.0 | 12.8 | 6.92 | 12 | 5.49 | 50.6 | 10.8 |
| Carleton | 35.6 | 12.3 | 4.66 | 14 | 3.55 | 48.7 | 7.1 |
| D.T. 208 | 36.5 | 12.8 | 5.54 | 10 | 3.98 | 46.6 | 6.8 |
| D.T. 122 | 36.8 | 12.8 | 5.25 | 16 | 3.72 | 47.9 | 6.4 |
| Mindum | 39.8 | 11.9 | 4.60 | 14 | 3.36 | 47.6 | 6.2 |
| D.T. 125 | 39.1 | 12.2 | 4.52 | 32 | 2.24 | 38.6 | 2.6 |
| Golden Ball | 39.5 | 11.3 | 4.39 | 41 | 1.62 | 38.3 | 2.4 |
| Mean | 37.5 | 12.3 | 5.13 | 20 | 3.42 | 45.5 | 6.1 |
| **Station Means** | | | | | | | |
| Swift Current | 46.0 | 10.5 | 5.15 | 20 | 3.54 | 48.1 | 7.8 |
| Leth-bridge | 33.2 | 13.5 | 5.72 | 21 | 3.95 | 47.1 | 7.3 |
| Morden | 40.5 | 12.8 | 5.34 | 23 | 3.59 | 45.8 | 6.5 |
| Melita | 30.8 | 13.6 | 5.29 | 18 | 3.61 | 45.7 | 6.5 |
| Saskatoon | 23.5 | 13.7 | 4.87 | 19 | 3.03 | 44.3 | 5.6 |
| Brandon | 55.8 | 11.0 | 5.35 | 19 | 3.54 | 44.8 | 5.2 |
| Indian Head | 32.5 | 10.8 | 4.16 | 20 | 2.68 | 42.5 | 3.6 |
| Mean | 37.5 | 12.3 | 5.13 | 20 | 3.42 | 45.5 | 6.1 |
| **Variance** | | | | | | | |
| Varieties | 28** | 2.17** | 5.60** | 939** | 10.87** | 170.8** | 57.3** |
| Stations | 816** | 14.86** | 1.73** | 17* | 1.26** | 23.8** | 14.0** |

[1] 14% moisture basis.

cerned in macaroni color are semolina pigment content and lipoxidase activity. There is, however, another unknown factor which exerts a lesser influence, for the correlation between macaroni pigment and macaroni color score for the 49 semolinas is only 0.87. Moreover, there are significant differences in mean color scores between two stations (Brandon and Melita) which are not a result of differences in either semolina pigment or lipoxidase activity. Nevertheless, the data as a whole support the hypothesis that the major factors affecting color score are semolina pigment and lipoxidase activity.

Analyses of covariance for semolina pigment (Ps), semolina lipoxidase (Ls), and macaroni pigment ($P_m$) yielded the following correlations:

| | D.F. | PsPm | LsPm |
|---|---|---|---|
| Total | 47 | 0.87** | −0.78** |
| Between varieties | 5 | 0.89** | −0.87** |
| Between stations | 5 | 0.97** | 0.21 |
| Interaction | 35 | 0.23 | 0.14 |

The very high between-stations correlation for the pigment contents of semolina and macaroni is a reflection of the previous observation that lipoxidase activity is little influenced by differences in environment. By contrast, the correlation involving lipoxidase (Ls) occurs between varieties and not between stations.

### Prediction Test for Small Samples of Semolina or Wheat

Considerable progress has been made during the last 20 years in the development of suitable laboratory methods of assessing macaroni-making quality both on a macro (1, 4) and a micro (3, 5, 8) scale. One of the major difficulties in reducing the amount of wheat required for a test has been the need for milling semolina to make either a disc test or a micro macaroni test. Accordingly, a prediction test for macaroni quality based on analyses of wheat seems desirable. From our observation of the usually close relationship between macaroni pigment content and macaroni color it seemed useful to investigate the possibility of predicting macaroni pigment content from analyses of ground wheat, or possibly of semolina. Determination of pigment in all these products is simple, accurate, and convenient, and represents a reproducible objective measurement. The development of the lipoxidase assay already outlined and the relations apparent in Table I led to preliminary experiments which yielded promising results.

Two parallel investigations have now been made using semolina and ground wheat as the test material. As would be expected, the results on ground wheat are not so good as those on semolina. For mill control work and on other occasions when rapid tests are needed and semolina is available, the semolina method provides an excellent and rapid estimate of quality. For plant breeders who have only small samples of new varieties to spare (of about 20 g.) the prediction test for wheat appears more useful.

*Materials.* The data for the semolina prediction test were obtained from 138 samples of semolina. These represent individual variety samples, composite variety samples, and grade samples made up as follows:

Mindum, Nugget, and an unnamed variety grown at four stations in Manitoba in 1951 (12 samples).

Carleton, Pelissier, Iumillo, Gaza, Chapinge, Beloturka, Heiti, and eight other varieties (all of poor quality), from the U.S.D.A. durum collection, grown in 1951 at Winnipeg (15 samples).

Mindum, Carleton, Nugget, Golden Ball, and three unnamed varieties grown at seven stations across Western Canada in 1952 (49 samples).

Mindum, Stewart, Carleton, Nugget, Golden Ball, Pelissier, and seven unnamed variety selections composited from six stations across Western Canada in 1951 (13 samples).

Mindum, Stewart, Carleton, Nugget, Pelissier, Golden Ball, and ten unnamed varieties composited from five stations across Western Canada in 1950 (16 samples).

Crop-year average samples of grades 2, 3, and 4 C. W. Amber Durum for 1950-1951 and 1951-1952; new-crop average samples of grades 2, 3, and 4 C.W.A.D. for 1951 and 2, 3, 4, and 5 C.W.A.D. for 1952; standard export samples of grades 2 and 3 C.W.A.D. for 1952 crop (15 samples).

Two commercial semolinas (two samples).

The samples used for the wheat prediction test were 93 wheat samples from among those listed above, namely:

Fifteen individual varieties from U.S.D.A. collection 1951 (15 samples).

Seven varieties grown at seven stations 1952 (49 samples).

Thirteen variety composites 1951 crop (13 samples).

Sixteen variety composites 1952 crop (16 samples).

*Semolina Prediction Test.* Lipoxidase activity and pigment content

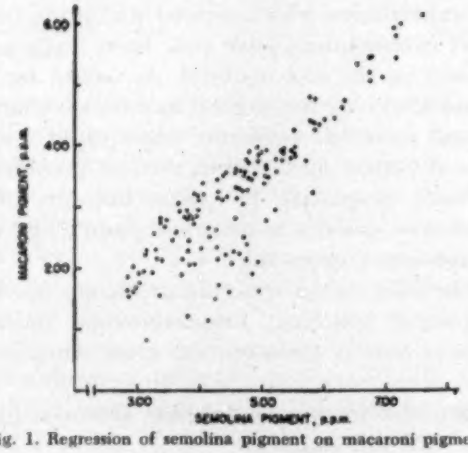


Fig. 1. Regression of semolina pigment on macaroni pigment.

were determined on the semolinas. Macaroni was processed from these and the pigment content of the ground macaroni was determined. Figure 1 shows the regression of semolina pigment on macaroni pigment for these 138 semolinas ($r = 0.87$). The values in the upper right-hand section of Fig. 1 are samples of Nugget or Nugget types. It is apparent why, in the days before Carleton, Stewart, or Nugget were developed, Binnington and Geddes (2) found only a relatively loose relationship between semolina or wheat pigment and macaroni color score.

The effect of including semolina lipoxidase activity in the regression is shown in Fig. 2. The correlation between calculated macaroni

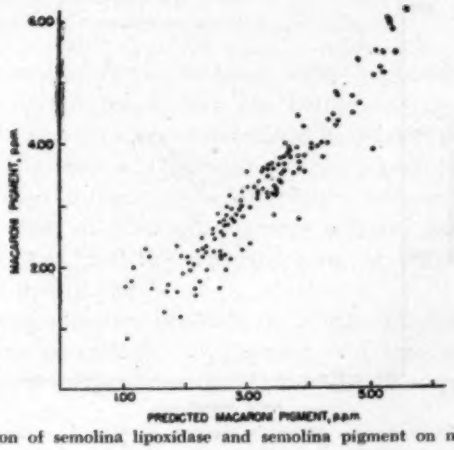


Fig. 2. Regression of semolina lipoxidase and semolina pigment on macaroni pigment.

pigment values and experimental values is now 0.95 and the standard error of estimate (s) is 0.35 p.p.m. Pertinent data on the regression study follow:

*Semolina prediction test ($N = 138$)*

*Correlations*

| | | |
|---|---|---|
| Semolina pigment *vs.* macaroni pigment | $r = 0.87$ | $s = 0.55$ |
| Semolina lipoxidase *vs.* macaroni pigment | $r = -0.68$ | |
| Semolina lipoxidase *vs.* semolina pigment | $r = -0.37$ | |

*Regression equation*

$P_m = 0.669 + 0.726\ Ps - 0.042\ Ls$ $\qquad s = 0.35$

where $P_m$ is macaroni pigment p.p.m.
Ps is semolina pigment p.p.m., and
Ls is lipoxidase activity of semolina in $\mu l.O_2/min./g.$

The negative correlation $r = -0.37$ between semolina pigment and semolina lipoxidase activity is highly significant and indicates that high lipoxidase activity is probably responsible, in some measure at least, for varieties with low semolina pigment. Thus the newer varieties of the Nugget type all have uniformly low lipoxidase values and exceptionally high pigment content.

*Wheat Prediction Test.* Wheat samples were ground and lipoxidase activity and pigment content were determined as with semolina. The 93 samples tested were all wheats which were milled to semolina and included in the semolina prediction test regression study; accordingly macaroni pigment data were available. The regression of wheat pig-

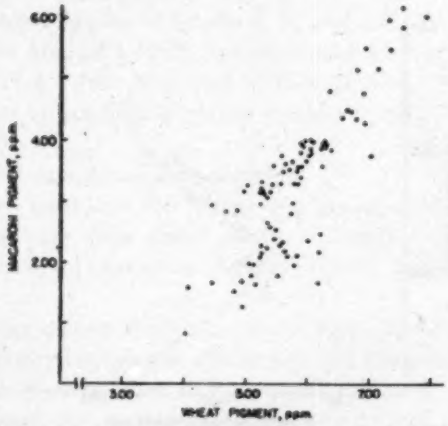


Fig. 3. Regression of wheat pigment on macaroni pigment.

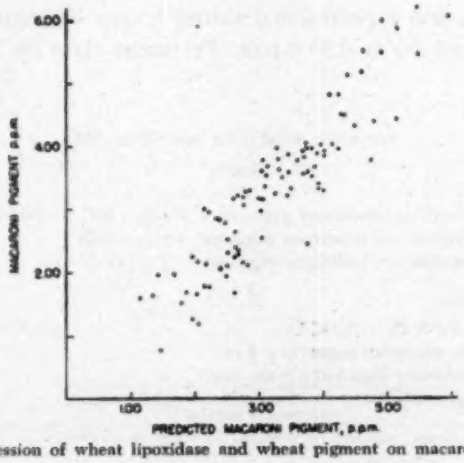


Fig. 4. Regression of wheat lipoxidase and wheat pigment on macaroni pigment.

ment on macaroni pigment is shown in Fig. 3 and of calculated macaroni pigment on experimental values in Fig. 4. Pertinent data on the regression study follow:

*Wheat prediction test ($N = 93$)*

| | | |
|---|---|---|
| *Correlations* | | |
| Wheat pigment *vs.* macaroni pigment | $r = 0.79$ | $s = 0.71$ |
| Wheat lipoxidase *vs.* macaroni pigment | $r = -0.77$ | |
| Wheat lipoxidase *vs.* wheat pigment | $r = -0.47$ | |
| *Regression equation* | | |
| $P_m = 0.114 + 0.844\ Pw - 0.043\ Lw$ | | $s = 0.48$ |
| where $P_m$ is macaroni pigment p.p.m., | | |
| Pw is wheat pigment p.p.m., and | | |
| Lw is lipoxidase activity of wheat in $\mu l.O_2$/min./g. | | |

For wheat the correlation between wheat pigment and macaroni pigment is somewhat lower than the corresponding correlation for semolina, while the negative correlations between lipoxidase activity and macaroni pigment and between wheat pigment and lipoxidase activity are somewhat higher. The correlation between calculated and experimental values of macaroni pigment is lower than for the semolina regression, 0.91, and the standard error of estimate correspondingly higher at 0.48 p.p.m.

This screening test may be made on wheat samples of 20 g., using standard routine procedures for pigment and lipoxidase determinations. If necessary, the methods could probably be scaled down to half this size.

## General Discussion

On the basis of objectively measurable properties, the newer varieties of durum wheat which have been licensed during the past 10 years differ from many of the older varieties in two respects: they are higher in pigment content, and lower in lipoxidase activity; as has been indicated, these properties may be related. The development of these better varieties has been the result of a tremendous amount of work on the part of both the plant breeder and the cereal laboratory which services his selections. It is now apparent that two of the most important factors which contribute to macaroni-making quality, at least in the durum areas of Canada and the United States, have been elucidated. By means of the screening test outlined above, it should now be possible to breed specifically for these factors and to eliminate unsatisfactory strains at a much earlier stage of develop-

ment. This is particularly important in view of the breeding program which must be undertaken to obtain resistance to the new races of stem rust which now threaten the durum crop with extinction. This laboratory has just begun a program of testing breeding stock from the world collection of the U. S. Department of Agriculture and it is already apparent that the majority of the varieties are likely to be high in lipoxidase activity and relatively low in pigment content. Lipoxidase values as high at $48\mu 1.0_2$/min./g. have been noted, as compared with Nugget which averages about 12, and this appears to be the rule rather than the exception.

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# INITIAL AND RESIDUAL EFFECTIVENESS OF SPOT FUMIGANTS IN ELEVATOR BOOTS[1,2]

F. L. WATTERS[3] AND B. N. SMALLMAN[4]


## ABSTRACT

Two proprietary fumigant mixtures, ethylene dichloride-carbon tetrachloride (3:1) and ethylene dibromide-chlorinated solvents (3:17), were compared with a new fumigant, hexachloropropene, against insects that infest elevator boots in flour mills. The proprietary fumigants gave 100% mortality of adults of the confused flour beetle, *Tribolium confusum* Duv., exposed for 1 day immediately after application, but failed to give appreciable mortalities 2 and 4 days later, respectively, when fresh lots of insects were exposed for 2-day periods. Hexachloropropene was ineffective the first day after application, but 2 days later gave 100% mortality. This effectiveness was maintained for 26 days when insects were exposed for 2-day periods, and for 59 days with 6-day exposures. Flour passed over hexachloropropene for periods up to 20 hours was free from taint or odor; baking quality was unaffected.


In cereal and other food processing plants, insects often occur in elevator boots and machines where "dead" stock accumulates. Two factors contribute to insect infestations in localized situations such as these: first, high reproduction associated with favorable environment in the machines; second, an influx of insects from incoming cereal stock and/or connecting spouts, conveyors, or elevators. The usual method of controlling such infestations is by the local application of fumigants to the infested stock. However, most of the commonly used fumigants evaporate rapidly and give no protection against reinfestation from outside sources. Because reinfestation of "dead" stock is a more or less continuous process (3), it appears that the important requirement for this type of fumigation is not so much that it should yield a high initial mortality as that it should yield a high toxicity over a longer period. Laboratory studies by Smallman (2) indicated that this objective could be attained with fumigants of relatively low vapor pressures and high toxicity. It was therefore postulated that fumigants of low vapor pressure were better suited to the special requirements for spot fumigation in flour mills than the highly volatile fumigants used for this purpose. The present study was designed to test this postulate under practical mill conditions.

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## Materials and Methods

Two proprietary fumigants were selected to represent compounds of high and moderately low vapor pressure. Ethylene dichloride has a vapor pressure of 80 mm. of mercury at 25°C. It has been widely used to control infestations in stored grain as well as in flour mills. It is usually mixed with carbon tetrachloride in the ratio 3 to 1 by volume to reduce fire hazard. Ethylene dibromide is less volatile than most of the commonly used fumigants; the vapor pressure is 12 mm. of mercury at 25°C. It is formulated as a 15% mixture with chlorinated solvents to give the proprietary fumigant Dowfume EB-15. This mixture is highly effective as a spot fumigant in elevator boots, the effectiveness being attributed to its high toxicity and low vapor pressure (3).

The insecticidal properties of hexachloropropene were first described by Brown (1). The residual toxicity of this compound in flour was demonstrated by Smallman (2) and was thought to result from its slow rate of volatilization and diffusion and because it was strongly sorbed by flour. Its vapor pressure is 0.3 mm. of mercury at 25°C. (1).

*Application.* Only boots of elevators carrying middlings, tailings, or low-grade flours were chosen for the experiments. From these, 20 elevator boots were selected at random from two flour mills. Five boots were assigned for treatment with each of three fumigants and five boots were allocated for controls. A single application was made when the mills were shut down.

The two proprietary fumigants, ethylene dichloride and ethylene dibromide, were each applied at the recommended dosage of 12 oz. per boot. The fumigants were poured into the boot stock through the hand-hole of the spout on one side and through the crack at the top of the slide on the other.

Boots treated with hexachloropropene were cleared of all stock. The hexachloropropene was applied to a double layer of blotting paper, 5 in. x 19 in., fastened to a strip of tin-foil to prevent loss by leakage. The dosage was 2 oz. per boot. The treated blotting paper was then placed in the boot and the stock returned.

*Assessment.* Treatments were assessed by bioassay with adults of the confused flour beetle, *Tribolium confusum* Duv. This method simplified the analysis of the results, because the normal variation in numbers of insects between boots and between cereal stocks was eliminated as a factor in the experiment. Other advantages were: (a) insects could be exposed in the treated boot for a certain period and

then withdrawn while the mill was running; (b) the boot stock was relatively undisturbed after withdrawal of test insects; (c) exposure of fresh lots of insects to the treated stock and their removal while the mill was running simulated introduction of insects from the moving stock or elevators.

The test insects were confined in wire-gauze envelopes 3 in. x 2½ in. About 11 g. of cereal stock from the treated boot and 25 adult beetles were placed in each envelope. Two envelopes were inserted in each boot, one from each side, by raising the slide slightly and pushing the envelope about 3 inches into the stock. This was done to ascertain whether there was any difference in effectiveness of the fumigant between sides due to rotation of the cups.

Effectiveness of each fumigant was based on the percentage mortality of 25 insects placed in each envelope. Insects were exposed initially for one day after the boot was treated to determine the immediate effectiveness of each fumigant. Residual effectiveness was assessed by subsequent 2-day exposures. The exposure period of insects to hexachloropropene was later increased to 6 days after it appeared to become less effective during the 2-day exposures. Mortality assessments were made after insects had been held on fresh flour for 4 days.

*Effect on Baking Quality.* Spot fumigants can be recommended only where no deleterious effect is imparted to the product to be fumigated. Both ethylene dichloride and ethylene dibromide have been declared safe for use on milled cereal products. However, no similar information is available for hexachloropropene. Baking tests were therefore carried out with flour that had been passed continuously for periods up to 20 hours over boot stock treated with hexachloropropene. An experimental elevator with a self-feeding spout was constructed for this purpose. Flour at the head was returned through a spout to the boot. Thus it was possible to pass the same flour through the treated boot a number of times.

## Results and Discussion

Figure 1 shows the initial and residual effectiveness of the three fumigants.

Ethylene dichloride was highly effective against insects exposed for one day immediately after treatment. When insects were introduced 1 day after treatment they suffered 60% mortality during the following 2 days. After 5 days the fumigant was ineffective under the conditions of the experiment.

Ethylene dibromide also gave a high initial mortality against

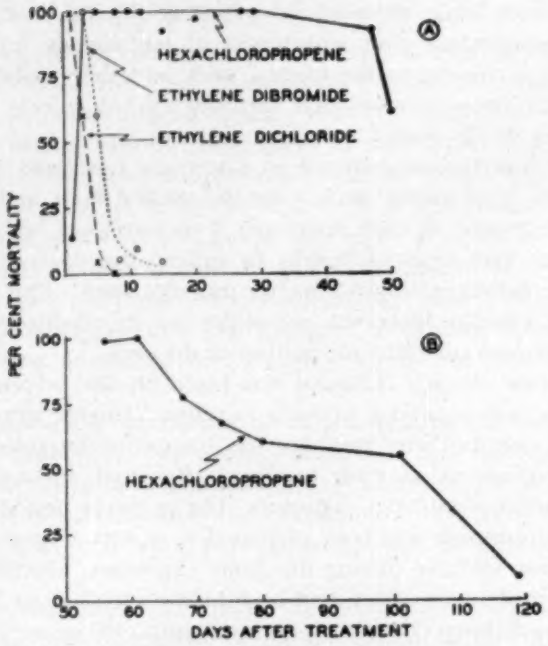


Fig. 1. Comparative effectiveness of spot fumigants in elevator boots against the confused flour beetle. A: initial 1-day exposure followed by 2-day exposures. B: 6-day exposures.

insects exposed for 1 day after treatment; but the effectiveness decreased rapidly after 5 days when insects were exposed for 2-day periods.

On the other hand, hexachloropropene gave a very low initial mortality the first day after treatment when insects were exposed for one day; but subsequent 2-day exposures gave nearly 100% mortality for 26 days. Forty-nine days after treatment mortality dropped to 65%. At this time, the exposure period was increased from 2 to 6 days and the mortality again approached 100% and was maintained at that level for an additional 13 days. Effectiveness decreased during subsequent 6-day exposures to 10% at 118 days after application.

There was no significant difference in mortality between the two sides of the treated boots. It was thought that more insects would be killed on the up-side of boots than on the down-side owing to greater draft from the movement of cups and consequent greater volatilization of the fumigant. There was a slightly higher mortality on the up-side than on the down-side of boots treated with ethylene dibromide and hexachloropropene, but these differences were not significant at the 5% level.

Loaves baked from flour that had been passed over hexachloropropene-treated boot stock showed no abnormal taint or odor. Flour that had been circulated continuously from spout to boot to elevator to spout for more than 0.5 hour generally produced larger loaves than the controls. This was attributed to aeration and attrition caused by continuous movement of the flour through the elevator.

The most striking difference among the three fumigants was their duration of effectiveness. The highly volatile fumigant ethylene dichloride became ineffective 3 days after application; ethylene dibromide, of moderately low vapor pressure, became ineffective after 5 days. In contrast, under comparable test conditions, hexachloropropene remained highly effective from 3 to 29 days after application. Effectiveness was prolonged beyond 49 days by increasing the exposure period from 2 to 6 days.

These results confirm the postulate that the special requirements for spot fumigation of elevator boots in flour mills can best be met with fumigants of low vapor pressure. It appears that where a residual action is desirable, a highly toxic fumigant of low volatiliy is preferable to fumigants of high volatility. The sorption of hexachloropropene by treated boot stocks and its gradual desorption over several weeks was probably the main factor in prolonging its effectiveness. The results indicate that in semi-open locations such as elevator boots low vapor pressure fumigants of high toxicity have an important application.

The wide difference in effectiveness between hexachloropropene and the higher vapor pressure fumigants, ethylene dichloride and ethylene dibromide, may be due in part to different methods of application. In the treatment of boots with the two proprietary fumigants the recommended method of pouring the fumigant down the handholes and inside of the slides was followed. This led to some loss by leakage from the bottom of the boot and slide so that not all of the fumigant reached the boot stock. On the other hand, the entire dosage of hexachloropropene was applied to a substrate and inserted beneath the boot stock. Hence a greater proportion of fumigant was available in the boot.

Under practical mill conditions the duration of the effectiveness of all treatments would probably be greater than that recorded here. It was necessary to raise boot slides to insert or remove envelopes containing test insects, and whenever this was done about half a pound of stock was forced out. This stock contained fumigant vapors and when withdrawn was immediately replaced by fresh stock from the spout. This would serve to decrease the concentration of fumigant

in the boot stock. All treated boots were subjected to this procedure.

Both mills were shut down for routine maintenance approximately once a week. It was expected that the effectiveness of hexachloropropene would drop during this period owing to the lack of air movement over the stock and a resultant decrease in rate of evaporation from the substrate. However, no change in effectiveness was observed during the weekly shutdown.

It is likely that insects in elevator boots would be exposed to a fumigant longer than the 2-day or 6-day period used in these studies. However, the exposure periods served to simulate the entry of insects into the boots at various times after treatment, and this permitted comparison of the immediate and residual effectiveness of the three fumigants tested.

**Acknowledgments**

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Hexachloropropene was made available through the courtesy of the Defence Research Board, Experimental Station, Suffield, Alberta.

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## BOOK REVIEWS

**The Production of Barley for Malting.** Prepared and Published by Muntona, Ltd., Bedford, England and Edward Fison, Ltd., Ipswich, England. 26 pp. Price: Gratis.

The Production of Barley for Malting is one in a series of monographs on malt and malt products prepared and distributed, free of charge, by these two malt processing and malting organizations. The brochure consists of 26 pages (5½" x 8½") and consequently the subject is sketchily treated. The purpose is apparently to make the English barley producers aware of the maltsters quality requirements and indicate to him how he can produce barley suitable for this market.

The 'Forword' states that 11 to 12% of the arable land in Great Britain is used in the production of barley and on which there are produced about 9½ million bushels, of which about 4 million bushels are manufactured into malt. Of this, 85½% is used for brewing and distilling and the balance for food and other purposes. The monograph then gives a quick glance at the life cycle of barley and indicates the maltsters requirements under such headings as: Capacity to Germinate, Moisture Content, Nitrogen Content, Shape and Size of Grain. It then states that malting quality and brewing quality may be quite distinct in that the latter is determined by the nitrogen and malt extract. A unique method of estimating the yield of extract is outlined.

The production of barley is dealt with under the headings: Crop Rotation, Choice of Seed, The Soil and Seed Bed, Manures and Fertilizers and Sowing Conditions. With some exceptions, these are similar to the recommendations in the United States and Canada.

The importance of variety is stressed and the contribution and problems of the plant breeder is emphasized. Some of the British and Western Europeon varieties are then described and classified. In one of the appendixes a method is outlined for determining a one-figure value for malting quality and then the value of the sample is depicted graphically for the production of such products as whiskey, vinegar, malt extract, lager, mild ale and pale ale.

In view of the fact that the publication deals entirely with British conditions and European varieties its recommendations will be of only academic interest to American and Canadian processors and producers.

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**Chemical Control of Insects.** By T. F. West, J. Eliot Hardy, and J. H. Ford. 211 pp. John Wiley & Sons: New York (1952). Price: $3.25.

The field of chemical control of insects is so rapid-moving and broad that an author or authors find a difficult task in summarizing the literature in a single book. The present book contains only 211 pages and thus the problem of adequate treatment of the subject is even more difficult. The result is that most topics must be treated in a rather cursory manner or specific topics treated more fully and other topics omitted or treated very briefly. The latter approach has been used in this book.

Actually, the chemicals and topics treated in more detail are old ones including nicotine, rotenone, arsenicals, pyrethrum, thiocyanates, and petroleum oil literature through 1947. DDT is treated in detail but in no case is the information brought up to date. All literature cited appears to be prior to 1948. There is no hint of the developing problem of housefly and mosquito resistance to DDT and chemically related chlorinated hydrocarbons. This difficulty was becoming rather widespread in 1951. No recent work with benzene hexachloride, chlordane or toxaphene is cited and aldrin, dieldrin, allethrin, new synergists, and new miticides are not mentioned. Of the organic phosphate compounds only the early work on hexaethyl tetraphosphate and parathion is presented. All the discussions reveal that developments in the field of study are not presented beyond the year 1947. The result is serious omissions in several phases of the subject. This is disappointing for a book published in and dated 1952.

One might say that the usefulness of the book would be to people who do not have time to keep up in the field of chemical control, but this would have to be qualified because the balance of the discussion of the chemicals is based on insecticides not in most prominent use in United States today. Workers in

foreign countries will probably find the book more useful particularly where older and naturally occurring insecticides are used to a large extent.

There are a few errors which I have noted such as the incorrect chemical formulae for ethylene dichloride (p. 43) and parathion (p. 111) and the spelling of *Nicotine aresket* as *araskat* (p. 54).

The field of chemical control of insects can be discussed profitably from many viewpoints by different authors and from this standpoint an additional book in the field has some merit. Other contemporary works bring us up to date, however, and in this respect the present volume has a distinct weakness.

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**Conductimetric Analysis at Radio Frequency.** By G. C. Blake. xvii–109 pp. 15x 22 cm. Chemical Publishing Co., New York, N. Y. Price $2.75.

The purpose of this brief book as stated in the preface is to describe the author's work in the field of conductimetric analysis at radio frequency and to supply the reader with all the information required to allow him to carry out such analyses. The author has done a good deal of work in the design and use of equipment suitable for use at a frequency of 1175 kc, and the electronic and accessory equipment are described in detail.

The book is strictly limited in scope, in accordance with the purpose as stated above, but will be of interest to research workers in the field of high frequency titration.

E. J. Meehan
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**La Biscuiterie, Patisserie et Boulangerie Industrielles et les produits de Regime.** By J. Kiger. 560 pp. Libarie des Sciences, Girardot and Cie, Paris, 1951. Price, fr. 3,600.

This book is divided into three main sections: (1) Raw materials, (2) Manufacture, and (3) Analysis. The first and largest section (386 pp.), which deals with the characteristics, methods of analysis and the properties of the raw materials is subdivided as follows: (a) Flours and starch containing materials (flours of wheat and succedanea, flour products, etc.), (b) Sugars and materials rich in sugars (glucose, levulose, honey, etc.), (c) Materials rich in lipids (butter, margarine, animal fat, etc.), (d) Leavening agents (biological and chemical), (e) Water, perfumes and dyes, (f) Complementary improvers (eggs, milk and milk products, commercial gluten, vegetable gums, organic acids, etc.), and (g) Vitamins.

The second section (119 pp.) describes the manufacture of dry cookies with different kinds of doughs (soft, hard, liquid, etc.), almond and egg-containing cookies, rusks, buns, pretzels, "bread" for special diets (e.g. for diabetics), etc. The last section (37 pp.) gives methods for the organoleptic, microscopic, microbiologic and chemical examinations of cookies, pastries, etc.

The book is well organized, contains a complete subject index and gives much interesting and useful information which is generally up-to-date. The chief criticism that can be made is the absence of any literature references. This severely limits a reader interested in detailed information especially on certain topics of a controversial nature. For instance, on p. 81, the author states categorically without citing any published work, that rancidity is favored by a moisture content less than 10% and that a moisture content between 10 and 12% is ideal for flour storage, an opinion by no means widely accepted by cereal chemists.

In spite of these limitations, the book is well worth reading and will be a useful source of information for those working in the field.

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## Cereal Chemistry

### EDITORIAL POLICY

**Cereal Chemistry** publishes scientific papers dealing with raw materials, processes, or products of the cereal industries, or with analytical procedures, technological tests, or fundamental research, related thereto. Papers must be based on original investigations, not previously described elsewhere, which make a definite contribution to existing knowledge.

**Cereal Chemistry** gives preference to suitable papers presented at the Annual Meeting of the American Association of Cereal Chemists, or submitted directly by members of the Association. When space permits, papers are accepted from other scientists throughout the world.

The papers must be written in English and must be clear, concise, and styled for **Cereal Chemistry.**

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## SUGGESTIONS TO AUTHORS

**General.** Authors will find the last volume of *Cereal Chemistry* a useful guide to acceptable arrangements and styling of papers. "On Writing Scientific Papers for Cereal Chemistry" (*Trans. Am. Assoc. Cereal Chem.* 6:1–22. 1948) amplifies the following notes.

Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, and all original drawings or photographs for figures. If possible, one set of photographs of figures should also be submitted. Originals can then be held to prevent damage, and the photographs can be sent to reviewers.

**Titles and Footnotes.** Titles should be specific, but should be kept short by deleting unnecessary words. The title footnote shows "Manuscript received . . ." and the name and address of the author's institution. Author footnotes, showing position and connections, are desirable although not obligatory.

**Abstract.** A concise abstract of about 200 words follows title and authors. It should state the principal results and conclusions, and should contain, largely by inference, adequate information on the scope and design of the investigation.

**Literature.** In general, only recent papers need be listed, and these can often be cited more advantageously throughout the text than in the introduction. Long introductory reviews should be avoided, especially when a recent review in another paper or in a monograph can be cited instead.

References are arranged and numbered in alphabetical order of authors' names and show author, title, journal, volume, first and last pages, and year. The list is given at the end of the paper. Reference numbers must invariably be cited in the text, but authors' names and year may be cited also. Abbreviations for the names of journals follow the list given in *Chemical Abstracts* **40:** I–CCIX. 1946.

**Tables.** Data should be arranged to facilitate the comparisons readers must make. Tables should be kept small by breaking up large ones if this is feasible. Only about eight columns of tabular matter can be printed across the page. Authors should omit all unessential data such as laboratory numbers, columns of data that show no significant variation, and any data not discussed in the text. A text reference can frequently be substituted for columns containing only a few data. The number of significant figures should be minimized. Box and side headings should be kept short by abbreviating freely; unorthodox abbreviations may be explained in footnotes, but unnecessary footnotes should be avoided. Leader tables without a number, main heading, or ruled lines are often usful for small groups of data.

Tables should be typed on separate pages at the end of the manuscript, and their position should be indicated to the printer by typing "(TABLE I)" in the appropriate place between lines of the text. (Figures are treated in the same way.)

**Figures.** If possible, all line drawings should be made by a competent drafts-

man. Traditional layouts should be followed: the horizontal axis should be used for the independent variable; curves should be drawn heaviest, axes or frame intermediate, and the grid lines lightest; and experimental points should be shown. Labels are preferable to legends. Authors should avoid identification in cut-lines to be printed below the figure, especially if symbols are used that cannot readily be set in type.

All drawings should be made about two to three times eventual reduced size with India ink on white paper, tracing linen, or *blue*-lined graph paper; with any other color, the unsightly mass of small grid lines is reproduced in the cut. Lettering should be done with a guide using India ink; and letters should be 1/16 to 1/8th inch high after reduction.

All original figures should be submitted with one set of photographic reproductions for reviewers, and each item should be identified by lightly writing number, author, and title on the back. Cut-lines (legends) should be typed on a separate sheet at the end of the manuscript. "Preparation of Illustrations and Tables" (*Trans. Am. Assoc. Cereal Chem.* **3**: 69–104. 1945) amplifies these notes.

**Text.** Clarity and conciseness are the prime essentials of a good scientific style. Proper grouping of related information and thoughts within paragraphs, selection of logical sequences for paragraphs and for sentences within paragraphs, and a skillful use of headings and topic sentences are the greatest aids to clarity. Clear phrasing is simplified by writing short sentences, using direct statements and active verbs, and preferring the concrete to the abstract, the specific to the general, and the definite to the vague. Trite circumlocutions and useless modifiers are the main causes of verbosity; they should be removed by repeated editing of drafts.

**Editorial Style.** A.A.C.C. publications are edited in accordance with *A Manual of Style*, University of Chicago Press, and *Webster's Dictionary*. A few points which authors often treat wrongly are listed below:

Use names, not formulas, for text references to chemical compounds. Use plural verbs with quantities. (6.9 g. were). Figures are used before unit abbreviations (3 ml.), and % rather than "per cent" is used following figures. All units are abbreviated and followed by periods, except units of time, which are spelled out. Repeat the degree sign (5°–10°C.). Place 0 before the decimal point for correlation coefficients ($r = 0.95$). Use * to mark statistics that exceed the 5% level and ** for those that exceed the 1% level; footnotes explaining this convention are no longer required. Type fractions on one line if possible, e.g., $A/(B + C)$. Use lower case for farinograph, mixogram, etc., unless used with a proper name, i.e., Brabender Farinograph. When in doubt about a point that occurs frequently, consult the Style Manual or the Dictionary.

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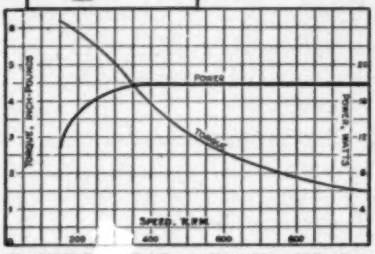


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